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ENZYME ACTION ON NORADRENALINE AND ADRENALINE

Studies on Bovine and Guinea Pig Tissues In Vitro with *Special Reference to Monoamine Oxidase*

MUNKSGAARD COPENHAGEN 1962

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INTRODUCTION

Noradrenaline, which is secreted at the sympathetic nerve ends as mediator of nerve impulses, and adrenaline, the hormone of the adrenal medulla, undergo rapid metabolism in certain tissues. The amount of adrenaline were observed to be very small in blood by PEKKARINEN, who used a chemical fluorimetric assay (1948). On the other hand, the amount of noradrenaline in some tissues was found to be high in bioassay (VON EULER 1956). The first fluorimetric assay also showed high noradrenaline content in spleen (PEKKARINEN & PITKÄNEN 1952). If infused into the circulation, noradrenaline and adrenaline rapidly disappear from it (PEKKARINEN 1948, LUND 1951) and only a small part of noradrenaline (1.5—4 %) and of adrenaline (0.5—1.0 %) is excreted into the urine, largest part being metabolized (VON EULER 1956). The main inactivation takes place in tissues, where noradrenaline liberated from the sympathetic nerve ends and adrenaline undergo enzymatic metabolism. Tyraminase enzyme, which was found by HARE (1928) and which has later been called monoamine oxidase, has for a long time been considered the principal inactivator of noradrenaline and adrenaline in the organism. The use of an effective monoamine oxidase inhibitor, iproniazid (ZELLER *et al.* 1952), has made it possible to throw more light upon the metabolism of noradrenaline and adrenaline. Another recently discovered enzyme, catechol-O-methyltransferase (AXELROD 1957), plays a role in the inactivation of noradrenaline and adrenaline in tissues. The occurrence and activity of these two enzymes differ from each other in different animal species (SPECTOR *et al.* 1959, WEINER 1960, SHIDEMAN & GOLDBERG 1961, CROUT *et al.* 1961).

The monoamine oxidase induced metabolism of noradrenaline and adrenaline in tissues has been studied indirectly using e.g. tyramine as substrate. The specific substrates noradrenaline and adrenaline have also been made use of more recently. The basic principles of the method (IISALO & PEKKARINEN 1958) as well as some preliminary observations (IISALO & PEKKARINEN 1954, IISALO 1957) have been published previously. The present study deals with the metabolism of noradrenaline and adrenaline as substrates in different bovine tissues *in vitro*. In addition to the oxidizing effect of monoamine oxidase on noradrenaline and adrenaline, it has also been tried to throw more light upon the probable part played by cytochrome oxidase in the metabolism of these substances.

EARLIER STUDIES

ELIMINATION OF NORADRENALINE AND ADRENALINE IN THE ORGANISM

1. Elimination in Blood and Tissues

The noradrenaline and adrenaline contents of the blood are very small The amounts published in numerous studies vary according to the method used in the determinations In different studies (PEKKARINEN 1948, LUND 1949, 1951 VON EULER 1956, WEIL-MALHERBE & BONE 1957, PRICE 1959, MANGER *et al* 1959, VENDSALU 1960) the average noradrenaline content is given as 0 1—4 1 µg/l and the average adrenaline content 0 0—1 1 µg/l in human peripheral plasma

In cat e g the medulla of only one adrenal gland secretes 0 034—0 160 µg/kg noradrenaline and 0 012—0 051 µg/kg adrenaline in one minute even when the animal is at rest (chloralose or pentobarbitone narcosis) (VON EULER 1956) Even 0 2 µg/kg/min noradrenaline or adrenaline infused intravenously increases the blood pressure and heart rate both in healthy persons (HEIKINHEIMO & PEKKARINEN 1952) and in patients suffering from shock (PEKKARINEN & ARO 1952) Adrenaline in the same dosage causes changes also in pulse pressure, blood sugar, blood lactic acid and serum inorganic phosphate (PEKKARINEN & HORTLING 1951) In addition to the secretion of adrenal glands there is also the secretion at the nerve ends Consequently the disappearance of noradrenaline and adrenaline from the blood is found to be quite rapid, It has been established that the rapid disappearance from the circulation into tissues takes place in all the capillaries (WEINSTEIN & MANNING 1937, PEKKARINEN 1948, LUND 1951 GOODALL *et al* 1959, AXELROD *et al* 1959c, HERTTING *et al* 1961) The first observations concerning the rapid elimination of adrenaline from the circulation were already made in the 19th century (OLIVER & SCHAFER 1894) The oldest studies dealing with this problem have been thoroughly reported on previously (PEKKARINEN 1948) On the basis of the latest investigations it has been calculated that the half life of endogenic noradrenaline and of adrenaline is 0 5 min —2 3 min in human plasma (COHEN *et al* 1959, VENDSALU 1960) Two minutes after an intravenous injection of radioactive H^3 adrenaline into a cat, a large amount

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In cat e g the medulla of only one adrenal gland secretes 0.034—0.160 μg/kg noradrenaline and 0.012—0.051 μg/kg adrenaline in one minute even when the animal is at rest (chloralose or pentobarbitone narcosis) (VON EULER 1956) Even 0.2 μg/kg/min noradrenaline or adrenaline infused intravenously increases the blood pressure and heart rate both in healthy persons (HEINE & PEKKARINEN 1952) and in patients suffering from shock (PEKKARINEN & ARO 1952) Adrenaline in the same dosage causes changes also in pulse pressure blood sugar, blood lactic acid and serum inorganic phosphate (PEKKARINEN & HORTLING 1951) *In addition* to the secretion of adrenal glands there is also the secretion at the nerve ends Consequently the disappearance of noradrenaline and adrenaline from the blood is found to be quite rapid, It has been established that the rapid disappearance from the circulation into tissues takes place in all the capillaries (WEINSTEIN & MANNING 1937, PEKKARINEN 1948, LUND 1951 GOODALL *et al* 1959, AXELROD *et al* 1959c, HERTTING *et al* 1961) The first observations concerning the rapid elimination of adrenaline from the circulation were already made in the 19th century (OLIVER & SCHAFER 1894) The oldest studies dealing with this problem have been thoroughly reported on previously (PEKKARINEN 1948) On the basis of the latest investigations it has been calculated that the half life of endogenic noradrenaline and of adrenaline is 0.5 min —2.3 *min* in human plasma (COHEN *et al* 1959, VENDSALU 1960) Two *minutes* after an intravenous injection of radioactive H^3 adrenaline into a cat, a large amount

of H^3 adrenaline and its metabolite (H^3 metadrenaline) was to be seen in the spleen, heart and various glandular tissues, whereas the amount was small in the muscles and negligible in the brain (AXELROD *et al.* 1959b, 1959c). The H^3 adrenaline content of the plasma decreased very rapidly during the first few minutes. Adrenaline added to the blood *in vitro* is, however, quite stable, there being no monoamine oxidase in the blood (BAIN *et al.* 1937, ZELLER 1951, BLASCHKO 1952).

The importance of tissues in the inactivation process of adrenaline has been emphasized for a long time (BAIN *et al.* 1937). The liver has been considered the most important organ inactivating adrenaline (TRENDELENBURG 1916, PAK 1926, MARKOWITZ & MANN 1929, TANI 1931, MACHII 1932, PHILPOT & CANTONI 1941, DAWES 1946, LUND 1957). Yet it is evident that no particular visceral organ is solely responsible for the inactivation of adrenaline (MARKOWITZ & MANN 1929). This is also confirmed by the finding (PEKKARINEN 1948) that adrenaline was eliminated from the circulation of test animals at the same rate both before and after the occlusion *of hepatic, renal and intestinal circulation. Ninety per cent of adrenaline* infused into the circulation may be eliminated from it in the skeletal muscles and in the skin too (CELANDER & MELLANDER 1955). Earlier enzyme studies have been reported on previously (IISALO & PEKKARINEN 1958).

The stores of endogenous noradrenaline and adrenaline in tissues are *many times greater than the amounts of these substances in the blood.* The tissue metabolism of noradrenaline and adrenaline secreted at the sympathetic nerve ends or released from the cell stores, is thus of greater importance than the occurrence of these substances in the blood. It has been established that different processes may be involved in the metabolism *of the "free" noradrenaline of the blood as compared* with that of the tissue noradrenaline (CROUT *et al.* 1961).

2. Excretion into Urine

In man the normal excretion of biologically active noradrenaline is 5.6—25.2 μg/24 hrs. and that of adrenaline 1.3—5.4 *μg/24 hrs.* (*KARKI 1956*). The mean noradrenaline excretion before major surgery was 24.3 μg/24 hrs. and the excretion of adrenaline 4.4 μg/24 hrs. (HAMMI *et al.* 1957). On the first three days after operations the mean excretions of noradrenaline were 51.1, 59.1 and 51.5 μg/24 hrs. and those of adrenaline 12.4, 13.5 and 8.8 μg/24 hrs. In a group of young persons *the normal excretion of noradrenaline* was 17.9 μg/24 hrs. and that of adrenaline 5.2 μg/24 hrs. (PEKKARINEN *et al.* 1960a). In patients with cardiac insufficiency the mean excretion of

noradrenaline was 18.5 μg/24 hrs and that of adrenaline 7 μg/24 hrs (PEKKARINEN *et al.* 1960 b). During the third trimester of normal pregnancy the mean excretion of noradrenaline is 18.9 μg/24 hrs and that of adrenaline 4.5 μg/24 hrs (CASTRÉN, to be published). The total excretion of noradrenaline and adrenaline in man is, as determined fluorimetrically, 54 μg/24 hrs on the average, of which adrenaline comprises 33 % (PITKÄNEN 1956). When infused intravenously, only 3 %—6 % of noradrenaline and 0.5 %—2 % of adrenaline are excreted into the urine unchanged (e.g. VON EULER & LUFT 1951, ELMADJIAN *et al.* 1956).

Adrenaline given orally is also excreted into the urine in a conjugated form, obviously as sulphate (RICHTER 1940, BEYER & SHAPIRO 1945, BEYER 1946, HARTUNG 1946). In the rabbit, noradrenaline has also been observed to be excreted as a conjugate with glucuronic acid (DODGSON *et al.* 1947, CLARK *et al.* 1951). Radioactive C^{14} DL-adrenaline increases the excretion of conjugated adrenaline in the rat if given orally but not if administered intravenously (SCHAYER *et al.* 1951). This finding *excludes conjugation* as the physiological pathway of the metabolism of adrenaline. The intestine and the liver are the most important conjugation places (BEYER 1956). Under physiological conditions a small amount of noradrenaline and adrenaline is excreted as conjugates with glucuronic acid in man. In the acid hydrolysis the biologically measured amount of noradrenaline and adrenaline increased 1.5—3 times from the original (VON EULER & ORWEN 1955, VON EULER 1956, ELMADJIAN *et al.* 1956).

Radioactive noradrenaline and adrenaline are excreted into the urine as 5 unidentified metabolites (SCHAYER 1951, SCHAYER *et al.* 1952, 1953, 1955). As metabolic products of noradrenaline and adrenaline in the urine there have more recently been found 3-methoxy-4-hydroxymandelic acid (ARMSTRONG *et al.* 1957), methoxynoradrenaline and methoxyadrenaline (AXELROD 1957, AXELROD *et al.* 1958 b, LABROSSE *et al.* 1958 b, SJOERDSMA *et al.* 1959), 3,4-dihydroxymandelic acid (GOODALL *et al.* 1958, ARMSTRONG & MCMILLAN 1959, ELMADJIAN 1959, VON EULER *et al.* 1959, LABROSSE *et al.* 1961) and 3-methoxy-4-hydroxyphenylglycol (AXELROD *et al.* 1959 b). After inhibiting catechol-O-methyltransferase with pyrogallol, large amounts of 3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylethylglycol were excreted into the urine of the cat (KIRSHNER 1960). Most studies on urinary metabolites have been made after infusing noradrenaline, adrenaline or their labelled forms or the material has consisted of patients suffering from pheochromocytoma. In man the amount of methoxyadrenaline excreted as the metabolite of endogenic noradrenaline is much smaller than the fraction of 3-methoxy-4-hydroxymandelic acid as compared with the relative proportion of these metabolite fractions in the excretion after the administration of exogenous noradrenaline (SJOERDSMA 1961).

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plasma of the rabbit there also occurs adrenaline dehydrogenase in addition to the enzymes of the phenolase type (IMAIZUMI & KAWAMOTO 1952) In the plasma, adrenaline dehydrogenase forms adrenalone, adrenaline's keto form, and when further oxidized, protocatechuic acid, too Adrenalone may be reduced reversibly into adrenaline Adrenalone is also met in the urine of guinea pigs after the administration of adrenaline (KITA 1958) Adrenaline dehydrogenase does not occur in the plasma of man or rabbit or in the extracts of rat liver, heart, spleen or brain (WEIL-MALHERBE & BONE 1957) Thus it has not been possible to establish that adrenaline dehydrogenase destroys adrenaline *in vivo*

C Monoamine Oxidase

The Properties and Distribution

Tyramine oxidase was first described by HARE (1928) Noradrenaline and adrenaline, too, were later observed to be inactivated as the substrates of this enzyme (BLASCHKO *et al* 1937 a) Tyramine oxidase, which was later called monoamine oxidase oxidizes noradrenaline and adrenaline into corresponding aldehydes while ammonia is liberated (KOHN 1937, RICHTER 1937) Aldehyde is then rapidly oxidized into dihydroxyphenyl glycolic acid by aldehyde oxidase (BLASCHKO *et al* 1937 a, BACQ 1949)

The activity of monoamine oxidase is usually determined manometrically by using tyramine or tryptamine as substrates and following the consumption of oxygen or the formation of ammonia (e g BLASCHKO *et al* 1937 a) Tissue suspensions and slices or mitochondrial preparations of the liver are used as the enzyme source Different enzyme inhibitors have been added to the manometric system to prevent the consumption of oxygen The formation of pigment in the tissues has also been used in the qualitative or quantitative determination of monoamine oxidase (BLASCHKO & HELLMAN 1953, FRANCIS 1953 EDER 1957, GLENNER *et al* 1957) To determine the monoamine oxidase activity in man *in vivo* 5-hydroxytryptamine has been given orally (SJOERDSMA *et al* 1958), and the decrease in the excretion of 5 hydroxy indolacetic acid caused by the enzyme inhibitor has been measured

In spite of several attempts it has not been possible to purify monoamine oxidase (KOHN 1937, ALLES & HEEGAARD 1943) and the uniformity of the enzyme has not been proved conclusively The uniformity of the enzyme is however, indicated e g by the fact that simultaneous substrates are not deaminated additively (BLASCHKO 1952), and that an effective inhibitor of the enzyme, iproniazid, inhibits the deamination of different substrates to the same extent (ZELLER *et al* 1955, PLETSCHER *et al* 1960)

3. Enzymatic Inactivation

A Cytochrome Oxidase and Phenolases

Cytochrome oxidase oxidizes the hydroquinone ring of adrenaline and converts it into a labile indophenol derivative adrenochrome In this process the side chain closes into a five ring and the adrenochrome is further converted into a dark brown melanin pigment (GREEN & RICHTER 1937 KEILIN & HARTREE 1938 BLASCHKO & SCHLOSSMANN 1938 1940) In the adrenaline inhibited smooth muscle tissues except the intestine there occurs an enzyme called catechol oxidase which turns adrenaline but not noradrenaline into adrenochrome *in vitro* (BACQ 1938) In animal tissues the amount of the phenolase is small and it has not any important biological function in the inactivation of adrenaline (BHAGVAT & RICHTER 1938) A similar reaction is seen in the oxidation of adrenaline which is usually called autoxidation (BLASCHKO & SCHLOSSMANN 1938) When muscular tissues are perfused with adrenaline and noradrenaline adrenochrome is formed by the cytochrome oxidase system (LUND 1951) This finding is supported by the observations of WAJZER (1946) In addition to the cytochrome oxidase there occurs another adrenaline oxidizing enzyme system in rat heart *in vitro* (HORNYKIEWICZ 1955) This enzyme is activated by heavy metals and inhibited by potassium cyanide as well Dopa oxidase metabolized adrenaline in the iris of pigmented rabbits whereas this did not occur in albino rabbits (ANGENENT & KOELLE 1952) α Naphtylthiourea reduced the consumption of oxygen by 50 %

As much as 50 μg adrenochrome has been reported to occur in one litre of human plasma under normal conditions (HOFFER 1958) Yet it has not been possible to verify this finding (SZARA *et al* 1958) Such reducing agents as the sulphydryl group (BLASCHKO 1952) and ascorbic acid (BLASCHKO 1952 IISALO & PEKKARINEN 1958) which are normally present in tissues protect the catechol amines from oxidation of the phenolase type and from autoxidation The formation of adrenochrome from adrenaline within the organism is also considered unlikely by SCHAYER *et al* (1953) Labelled adrenochrome is excreted into the urine as metabolites which differ from those of adrenaline The oxidation of adrenaline in human plasma is in correlation with the coeruloplasmin content of the plasma (HOLMBERG & LAURELL 1951) Ferritine also causes an oxidation of the phenolase type of adrenaline *in vitro* (GREEN *et al* 1956)

B Adrenaline Dehydrogenase

The oxidation product of the aldehyde corresponding to adrenaline is found in the urine of the rabbit (WEINSTEIN & MANNING 1937) In the

Table 1 (contin.)

Iproniazid	liver from rat, guinea pig, cat and mouse, inhibition of N and A metabolism	+	10 [illegible]—10 [illegible] M	ZELLER *et al.* 1955, GRIESEMER & WELLS 1956, CORNE & GRAHAM 1957
		+	10 [illegible]—10 [illegible] M	SIEBER *et al.* 1957
	rat brain, inhibition of N metabolism; bovine heart muscle, small intestine and uterus	+	10 [illegible]—10 [illegible] M	IISALO 1957
	homogenates, inhibition of N and A metabolism; potentiation of N and A constriction in rabbit aortic strips	−	10 [illegible] M	PLETSCHER *et al.* 1955
Choline p-tolylether	cat, rat and guinea pig liver, inhibition of N and A metabolism	+	1—80 μg/ml	GRIESEMER & WELLS 1956, BROWN & HEY 1956, CORNE & GRAHAM 1957
Atebrin® (quinacrine)	tissue extracts from dog, rabbit, guinea pig and mouse, inhibition of A metabolism	+		ALLEGRETTI & VUCADINOVIC 1950
Benadryl® and Anergen®	bovine liver homogenate, inhibition of A metabolism	+	20—200 mg/ml	KURIAKI & UCHIDA 1955
Ethylurethane	guinea pig and rat liver, inhibition of A metabolism	+		BLASCHKO *et al.* 1937 b
Procaine hydrochloride	liver slice, inhibition of A metabolism	+	100—1000 μg/ml	PHILPOT 1940, ORZECHOWSKI 1941, BROWN & HEY 1956
Pentothal sodium®	acetone dried guinea pig liver, inhibition of A metabolism	+	0.05—10 mg/ml	MALAFAYA BAPTISTA *et al.* 1955
Tranylcypromine	rat heart, inhibition of N metabolism	+		SHIDEMAN & GOLDBERG 1961
	cat heart, inhibition of N metabolism	−		SHIDEMAN & GOLDBERG 1961

Table 1

In vitro experiments with monamine oxidase (MAO) inhibitors and using noradrenaline (N) and adrenaline (A) as substrates

Inhibitor	Enzyme source and MAO induced change in N or A metabolism	MAO inhibition (+) or potentiation (+) of biological response No response (—)	Inhibitor concentration	Reference
Cocaine	Liver extracts and slices, inhibition of A metabolism	+	300—1000 µg/ml	Miyake 1952, Brown & Hey 1956
Ephedrine	cat liver, inhibition of A metabolism	+	10^{-2} M	Richter & Tingey 1939
	guinea pig liver, inhibition of A metabolism	+		Orzechowski 1941
	bovine and swine liver, inhibition of A metabolism	+	2—40 µg/ml	Heim 1947
	liver slice, inhibition of A metabolism	+	30—300 µg/ml	Brown & Hey 1956
	rat brain, inhibition of N metabolism	+	10^{-2} M	Shore *et al.* 1957
	potentiation of A induced constriction in carotid artery from swine	+		Smith & Alpert 1954
	in carotid artery from dog	—		Smith & Alpert 1954
Amphetamine	bovine and swine liver, inhibition of A metabolism	+	4—40 µg/ml	Blaschko 1940; Heim 1947
	guinea pig liver, inhibition of A metabolism	+	2×10^{-2} M	Orzechowski 1941
	liver slices, inhibition of A metabolism	+	10—100 µg/ml	Brown & Hey 1956
	bovine heart, inhibition of N and A metabolism	+	3.5—20 mM	Iisalo & Pekkarinen 1954, 1958
Octylalcohol	liver extracts from man, rat, guinea pig, cat and mouse, inhibition of A metabolism	+	10^{-2} M—saturated	Blaschko *et al.* 1937 [illegible]

Table 1 (cont.)

Iproniazid	liver from rat, guinea pig, cat and mouse, inhibition of N and A metabolism	+	10^{-5}—10^{-4} M	Zeller *et al.* 1955, Griesemer & Wells 1956, Corne & Graham 1957
	rat brain, inhibition of N metabolism	+	10^{-3}—10^{-4} M	Shore *et al.* 1957
	bovine heart muscle, small intestine and uterus homogenates, inhibition of N *and* A metabolism	+	10^{-4}—10^{-5} M	Iisalo 1957
	potentiation of N and A constriction in rabbit *aortic strips*	−	10^{-5} M	Furchgott *et al.* 1955
Cholin-p-tolylether	cat, rat and guinea pig liver, inhibition of N and A metabolism	+	3—80 μg/ml	Griesemer & Wells 1956, Brown & Hey 1956, Corne & Graham 1957
Atebrin® (quinacrine)	tissue extracts from dog, rabbit, guinea pig and *mouse*, inhibition of A metabolism	+		Allegretti & Vucadinovic 1950
Benadryl® and Anergen®	bovine liver homogenate, inhibition of A metabolism	+	20—200 mg/ml	Kuriaki & Uchida 1955
Ethylurethane	guinea pig *and* rat liver, inhibition of A metabolism	+		Blaschko *et al.* 1937 b
Procaine hydrochloride	liver slice, inhibition of A metabolism	+	100—1000 μg/ml	Philpot 1940, Orzechowski 1941, Brown & Hey 1956
Pentothal sodium®	*acetone dried* guinea pig liver, inhibition of A metabolism	+	0.05—10 mg/ml	Malafaya Baptista *et al.* 1955
Tranylcypromine	rat heart, inhibition of N metabolism	+		Shideman & Goldberg 1961
	cat heart, inhibition of N metabolism	−		Shideman & Goldberg 1961

Table 1

In vitro experiments with monamine oxidase (MAO) inhibitors and using noradrenaline (N) and adrenaline (A) as substrates

Inhibitor	Enzyme source and MAO induced change in N or A metabolism	MAO inhibition (+) or potentiation (+) of biological response No response (—)	Inhibitor concentration	Reference
Cocaine	Liver extracts and slices, inhibition of A metabolism	+	300—1000 µg/ml	MIYAKE 1952, BROWN & HEY 1956
Ephedrine	rat liver, inhibition of A metabolism	+	10^{-3} M	RICHTER & TINGEY 1939
	guinea pig liver, inhibition of A metabolism	+		OPZECHOWSKI 1941
	bovine and swine liver, inhibition of A metabolism	+	2—40 µg/ml	HEIM 1947
	liver slice, inhibition of A metabolism	+	30—300 µg/ml	BROWN & HEY 1956
	rat brain, inhibition of N metabolism	+	10^{-3} M	SHORE *et al* 1957
	potentiation of A induced constriction in carotid artery from swine	+		SMITH & ALPERS 1954
	in carotid artery from dog	—		SMITH & ALPERS 1954
Amphetamine	bovine and swine liver, inhibition of A metabolism	+	4—40 µg/ml	BLASCHKO 1940, HEIM 1947
	guinea pig liver, inhibition of A metabolism	+	2×10^{-3} M	OPZECHOWSKI 1941
	liver slices, inhibition of A metabolism	+	10—100 µg/ml	BROWN & HEY 1956
	bovine heart, inhibition of N and A metabolism	+	3.5—20 mM	IISALO & PEKKARINEN 1954, 1958
Octylalcohol	liver extracts from man, rat, guinea pig, cat and mouse, inhibition of A metabolism	+	10^{-3} M—saturated	BLASCHKO *et al.* 1937

Inhibition *in vitro*

The tissue metabolism of noradrenaline and adrenaline *in vitro* has been studied by using e g different mammalian liver or brain homogenates as the enzyme preparates A number of specific enzyme inhibitors have been made use of (Table 1) The effect of monoamine oxidase activity on noradrenaline and adrenaline *in vitro* has also been followed in arterial walls (SMITH & ALPERT 1954 FURCHGOTT *et al* 1955) and in the heart muscle (SHIDEMAN & GOLDBERG 1961) Table 1 shows results from experiments *in vitro* where noradrenaline and adrenaline have been employed as substrates Studies with other biogenic amines as substrates have been excluded, even though noradrenaline and adrenaline metabolism may have been dealt with in them

Iproniazid (ZELLER *et al* 1952) and other hydrazine derivatives have proved to be the most effective inhibitors of monoamine oxidase Iproniazid produces a progressive and irreversible inhibition of monoamine oxidase (ZELLER *et al* 1955 DAVISON 1957, HESS *et al* 1958) However the presence of substrate introduces reversibility into the enzyme-inhibitor complex (GLUCKMAN & MARRAZZI 1958) The reaction involved requires oxygen and it is supposed that the irreversible inhibition results from the dehydrogenation of iproniazid in the active centre of the enzyme (DAVISON 1957) The inhibition of monoamine oxidase by iproniazid is greatly intensified by the presence of *potassium cyanide* The importance of monoamine oxidase as the noradrenaline and adrenaline *inactivating* enzyme is mainly based on tests with specific enzyme inhibitors The amounts of enzyme inhibitors needed in the earlier experiments *in vitro* have often been considerably greater than the probable contents *in vivo* (FURCHGOTT 1955)

Inhibition *in vivo*

Tables 2 and 3 represent *in vivo experiments* where the changes in noradrenaline and adrenaline reactions have been *considered* to be due to monoamine oxidase inhibition GADDUM and KWIATKOWSKI (1938) observed the *adrenaline* induced vasoconstriction to be potentiated by 1×10^{-5} M ephedrine when perfusing a rabbit's ear Since ephedrine was known as a monoamine oxidase inhibitor *in vitro* (BLASCHKO *et al* 1937 a) they supposed that the destruction of the substance liberated at the adrenergic nerve ends was prevented by the inhibition of this *enzyme* A similar mechanism was supposed to be behind the cocaine induced *potentiation* of the biological reactions produced by adrenaline or by sympathetic stimulation In the perfusion test cocaine, amphetamine and desoxyamphetamine potentiate however the vasoconstriction *caused* by nor

Monoamine oxidase is thermolabile but quite stable at a low temperature (Davison 1958). The optimum pH of monoamine oxidase varies according to the enzyme preparate and substrate (Alles & Heegaard 1943, Pletscher *et al* 1960). For tyramine the optimum pH is 7.1—7.4 (Malafaya Baptista *et al* 1957, Davison 1958). When using adrenaline as a substrate its spontaneous oxidation increases to such an extent, when passing from pH 7.4—7.5 in the alkaline direction, that the establishing of the optimum pH is rendered impossible (Malafaya Baptista *et al* 1957, Iisalo & Pekkarinen 1958, Leeper *et al* 1958).

Monoamine oxidase contains an active sulphydryl group, which is necessary for its activity (Friedenwald & Herrmann 1942, Singer & Barron 1945, Sourkes 1958, Manukhin 1958, Agin 1959). Certain diamines are oxidized by the enzyme, for which reason the name amine oxidase has been suggested instead of monoamine oxidase (Blaschko 1952). On the other hand the amine oxidases that are called monoamine oxidases form a comparatively uniform group, which, contrary to diamine oxidases is semicarbazide resistant (Zeller 1959). Monoamine oxidase has only one kind of receptor in its active centre unlike diamine oxidase, which has receptors of two different kinds (Zeller *et al* 1956, Zeller 1959).

Noradrenaline and adrenaline act as substrates of monoamine oxidase although the rate of their metabolism is clearly smaller than that of tyramine, tryptamine or dopamine (e.g. Blaschko *et al* 1937 a, Weiner 1960, Pletscher *et al* 1960). However, the affinity of monoamine oxidase to different substrates does not vary considerably (Blaschko *et al* 1937 a).

Monoamine oxidase has been found in all vertebrates (Blaschko 1953) as well as in invertebrates (Blaschko & Hope 1957). The enzyme occurs in particularly large quantities in the liver, but it has also been found in almost every other mammalian tissue as well (Bhagvat *et al* 1939). It has been found in the digestive tract with its glands, in the kidneys, uterus, placenta, lungs, male genitals and even in the adrenal cortex and medulla. In addition to these it also occurs in the brain and the autonomic ganglions of the central nervous system, in the heart, blood vessels — arteries in particular — iris and nictitating membrane (Pugh & Quastel 1937, Blaschko *et al* 1937 a, Kohn 1937, Langemann 1944, Schapira 1945, Thompson & Tickner 1949, 1951, Blaschko 1952, 1953, Werle & Roewer 1952). Yet it does not occur in the blood (Zeller 1951, Blaschko 1952). Cellular *monoamine* oxidase is *present* in cytoplasm granules, mostly in mitochondria but also in the smaller microsomes (Cotzias & Dole 1951, Hawkins 1952, Blaschko 1953). There exists no selective connection between the occurrence of monoamine oxidase and adrenergic nerves (Koelle & Valk 1954, Arioka & Tanimukai 1957, Eder 1957, Glenner *et al* 1957).

Table 2 (contin.)

β-phenylisopropylhydrazine	*retardation* of A elimination in whole mouse	—	0.2 mg/kg	UDENFRIEND *et al.* 1959
	increase in N content in rabbit, rat and cat brain	+	1—20 mg/kg	SPECTOR *et al.* 1958, BRODIE *et al.* 1959, CARLSSON *et al.* 1959 a, KÄRKI *et al.* 1962
	increase in N content in rat brainstem	—		ROWE 1959
	potentiation of *cardiovascular responses* to N	—		GOLDBERG & SJOERDSMA 1959
	increase in endogenous N and A excretion in man	—		SJOERDSMA *et al.* 1959
Nialamide	*increase* in N content in rat brain	+	10—200 mg/kg	CARLSSON *et al.* 1959 a, MUSCHOLL 1959 a, ROWE 1959
Harmaline and other harmala alkaloids	increase in N content in rat *brain* and heart and cat *plasma*	+	5—10 mg/kg	MUSCHOLL 1959 b, PLETSCHER & BESENDORF 1959, PLETSCHER *et al.* 1959, EAKINS & LOCKETT 1961
	potent*iation of card*iovascular responses to N	—		GOLDBERG & SJOERDSMA 1959
Tranylcypromine	*increase* in N content in rat brain	+	5—20 mg/kg	CARLSSON *et al.* 1959 a, GREEN & ERICKSON 1960
4-phenyl-2-butyl-hydrazine	potentiation of cardiovascular *responses to* N	—		GOLDBERG & SJOERDSMA 1959
2-benzyl-1-picolinylhydrazine	potentiation *of cardio*vascular responses to N	—		GOLDBERG & SJOERDSMA 1959
Methylene blue	potentiation of blood pressure reaction *produced* when injecting A in v. portae	+		PHILPOT & CANTONI 1941

Table 2

Potentiation of noradrenaline (N) and adrenaline (A) reactions and inhibition of noradrenaline and adrenaline metabolism by monoamine oxidase (MAO) inhibitors *in vivo* (Iproniazid in table 3.)

Inhibitor	Species and reaction observed	Change in biological reaction (+) No change (—)	Inhibitor concentration	Reference
Cocaine	potentiation of A induced nictitating membrane and blood pressure response	+		FROHLICH & LOEWI 1910, GADDUM & KWIATKOWSKI 1938
	increase in N and A contents in cat heart, liver and spleen	—		VON EULER & HELLNER BJORKMAN 1955
Ephedrine	potentiation of A induced nictitating membrane and blood pressure response in cat	+		DAWES 1946
	increase in N and A contents in cat heart, liver and spleen	—		VON EULER & HELLNER BJÖRKMAN 1955
	potentiation of N and A induced vaso constriction in perfused rabbit ear	+	10 μg/ml	GADDUM & KWIATKOWSKI 1938, BURN & ROBINSON 1951
	retardation of injected A elimination in rabbit circulation	—		PEKKARINEN 1948
Amphetamine	increase in N and A contents in cat heart, liver and spleen	—	5—20 mg/kg	VON EULER & HELLNER BJÖRKMAN 1955
	increase in N content in rat brain	—	10 mg/kg	CARLSSON *et al* 1959 a
	retardation of A elimination in whole mouse	—		AXELROD & TOMCHICK 1960
Propamidine	increase in N and A contents in cat heart	+	6—30 mg/kg	VON EULER & HELLNER BJÖRKMAN 1955
	increase in N and A contents in liver and spleen	—		VON EULER & HELLNER BJORKMAN 1955
Cholin p tolylether	changes in metabolite distribution in rat urine chromatography after N and A injection	+	20—100 mg/kg	SCHAYER *et al* 1954, 1955
	increase in N and A contents in cat heart, liver and spleen	—		VON EULER & HELLNER BJÖRKMAN 1955
	potentiation of A induced vasoconstriction in perfused rabbit ear	[illegible]		

β]henyl so-(repyl))draz ne	retar lat on of A el m nat on n hole nouse	—	ng/kg	U ENFR END et al 1959
	ncrease in N content n rabl t rat an l cat l ra	+	1—?0 mg/kg	SPECTOR et al 1958 BRODIE et al 1959 CARLSSON et al 1959 a KÄRKI et al 1962
	n rease n N content n rat l ra nstem	—		RO VE 1959
	pot nt at on of car l o ascular responses to N	—		GOLDBF G & SJOE DSMA 1959
	increase n en log nous N an l N excret on n man	—		SJOF DSMA et al 1959
N alan le	n reas n N content n rat l ra n	+	10—?00 mg/kg	CARLSSON et al 1959 a MUSCHOLL 1959 a RO VE 1959
Harmal ne and tl er l armala al alo ls	ncrease in N c ntent n rat l rain and heart an l cat plasn a	+	5—10 mg/kg	M SC OLL 1959 b PLETSCHER & BESEN ORF 1959 PLETSCHER et al 1959 F AK NS & LOCKETT 1961 GO DBE G & SJOEI DSMA 1959
	] o ent at on of card o ascular responses to N	—		
Tranyleyf romine	ncrease n N content t rat bra n	+	5—?0 mg/kg	CARLS ON et al 1959 a GREEN & FRICKSON 1 0
4]]enyl ? l utyl l y lraz ne	potent at on of ar l o a cular responses to N	—		GOLD F G & SJOERDSMA 1959
benzyl 1 picol nyl l ydraz ne	] otent at on of car l o ascular responses to N	—		GOLD F G & SJOERDSMA 1959
Methylene blue	] otent at on of l loo l pressure react on produced hen nject ng A n v portae	+		PHILPOT & CANTONI 1941
Atel r n ® (quinacr ne)	nl l t on of A metal ol sm in t ssue l on ogenates	+	0 3 g/ral b t	ALLF ETTI & VUKADINOVIC 1950

Table 3

Potentiation or change of *noradrenaline* (N) and adrenaline (A) responses by monoamine oxidase (MAO) inhibitor iproniazid *in vivo*

Species and reaction *observed*	Change in biological reaction (+) No change (—)	Iproniazid concentration	Reference
Potentiation of N or A induced blood pressure or nictitating membrane response	— —	*0.18 mmol/kg* 40 mg/kg	GRIESEMER *et al* 1953, BURN *et al* 1954, BALZER & HOLTZ 1956, CORNE & GRAHAM 1957, VARAGIĆ 1958
Potentiation of nictitating membrane response induced by sympathetic stimulation or by N and A	—	0.4—0.5 mmol/kg	KAMIJO *et al* 1956
Inhibition of A metabolism in rat tissues	+	5×10[illegible] M	*ZELLER et al* 1955
Inhibition of A *metabolism* in guinea pig and cat liver homogenates	+	50 mg/kg	GRIESEMER & WELLS 1956
Increase in A toxicity in guinea pig	+		REBHUN *et al* 1954, BOROWITZ & NORTH 1959
Increase in endogenous N and A excretion in guinea pig	+	10—15 mg/day	PEKKARINEN *et al* 1960 b
Increase in endogenous N and A excretion in rat Increase in endogenous N and A excretion in man	— —	*150 mg/kg* *100 mg/day*	CRAWFORD & LAW 1958 CARLSSON *et al* 1959
Increase in *exogenous* N and A excretion in cat	+	10—20 mg/kg	CORNE & GRAHAM 1957
Increase in exogenous N and A *excretion in rat*	+	150 mg/kg	CRAWFORD & LAW 1958
Increase in exogenous N excretion in man	±	10 mg/kg	FRIEND *et al* 1958, ROSEN & GOODALL 1961

Table 3 (contin.)

Change *in rat* urine chromatography after N and A *injections*	+	200 mg/kg	SCHAYER *et al* 1953, 1955
Decrease in 3 methoxy 4 hydroxymandelic acid and 3 4 dihydroxymandelic acid *excretion* after infusion of A in man	+	50—200 mg/day	GOODALL *et al* 1958, RESNICK et al 1958, VON STUDNITZ 1959, GOODALL 1959
Decrease in 3 methoxy 4 *hydroxy*mandelic acid and 3 methoxy 4 hydroxy*phenyl*ethylglycol and increase in A excretion *after exogenous* A in cat	+		KIRSHNER 1960
Decrease in 3 methoxy 4 hydroxymandelic acid *and increase* in methoxyadrenaline excretion after exogenous A *in rat*	+		AXELROD 1959 a
Increase in N content in *rabbit* brain and inhibition of reserpine or raunescine induced *decrease* in N content in rabbit brain, heart and *adrenals*	+	50 mg/kg	SHORE *et al* 1957, BERTLER *et al* 1957, PAASONEN & KÄRKI 1959
Increase in N content in rat brainstem	—		ROWE 1959
Increase in catecholamine *content* in rabbit, rat and mouse brain	+	10—200 mg/kg	PLETSCHER 1957, SPECTOR *et al* 1958, CARLSSON *et al* 1959, EHRINGER *et al* 1960, GREEN & ERICKSON 1960, PAASONEN & KÄRKI 1959
Increase in N content in rat heart, spleen, intestine, kidney and *liver*	+	50—100 mg/kg	PEKKARINEN *et al* 1958
Increase in N content in guinea pig and mouse heart	+	100 mg/kg	PLETSCHER 1958
Increase in N content in *guinea* pig heart	—	100 mg/kg	MUSCHOLL 1959 a
Prolongation in metabolism of injected A in whole mouse	—	3 mg/mouse	UDENFRIEND *et al* 1959

adrenaline and adrenaline (BURN 1952) less than ephedrine does. Consequently it has been suggested that the supersensitivity to cocaine and like compounds should not be due to monoamine oxidase inhibition (FURCHGOTT 1955). α-Cocaine, a structural isomer of cocaine, inhibits monoamine oxidase as effectively as cocaine *in vitro*, but is unable to potentiate the reactions of adrenaline (FOSTER *et al.* 1955). The cocaine induced supersensitivity is connected with the adrenaline content of blood (TRENDELENBURG 1959). It may be due to the changes taking place in the uptake-release mechanism of noradrenaline in tissues (BEJRABLAYA *et al.* 1958, WHITBY *et al.* 1960, MUSCHOLL 1960, LINDMAR & MUSCHOLL 1961, HERTTING *et al.* 1961).

The sensitization of the nictitating membrane in the cat to noradrenaline and adrenaline after sympathetic denervation (BURN & HUTCHEON 1949) was thought to be due to the decrease in the amount of monoamine oxidase in the organ (BURN & ROBINSON 1952, ROBINSON 1952, BURN 1953). This decrease in the monoamine oxidase content could not, however, be demonstrated (ARMIN *et al.* 1953, BURN *et al.* 1954). There is no histochemical evidence of the localization of the monoamine oxidase enzyme at the adrenergic nerve ends (KOELLE & VALK 1954). The supersensitivity of the sympathetically innervated organs after denervation may be due to the release of noradrenaline stores within these organs (BURN & RAND 1959).

Although iproniazid is greatly superior to ephedrine or cocaine as an inhibitor of monoamine oxidase (ZELLER *et al.* 1952) it does not, however, potentiate the vasopressor or nictitating membrane response of noradrenaline and adrenaline to any considerable degree (GRIESEMER *et al.* 1953, BURN *et al.* 1954, BALZER & HOLTZ 1956, CORNE & GRAHAM 1957). On the other hand, the biological reactions of tyramine are clearly potentiated by iproniazid (CORNE & GRAHAM 1957). However, iproniazid when injected *in vivo*, as well as cholin *p*-tolylether, β-phenylisopropylhydrazine and other recent monoamine oxidase inhibitors, inhibit the property of brain, liver and kidney to metabolize adrenaline or noradrenaline (e.g. GRIESEMER & WELLS 1956, CORNE & GRAHAM 1957, SPECTOR *et al.* 1958).

The slight iproniazid induced potentiation of reactions produced by adrenergic nerve stimulation is in all probability not due to monoamine oxidase inhibition (KAMIJO *et al.* 1956).

In an anesthetized cat iproniazid increases the excretion of free infused adrenaline into the urine from 2.54 ± 0.27 % to 5.2 ± 0.31 % and that of noradrenaline from 3.7 ± 0.15 % to 5.4 ± 0.35 % (CORNE 1956). In the rat this increase also takes place (CRAWFORD & LAW 1958) but iproniazid does not add the excretion of endogenous noradrenaline and adrenaline

In guinea pigs the excretion of endogenous noradrenaline and adrenaline are clearly increased by iproniazid (PEKKARINEN *et al* 1960) In man no increase in the noradrenaline excretion is observed during iproniazid treatment (CARLSSON *et al* 1959) which might be due to the small therapeutic dose of iproniazid This finding also refers to the excretion after *infusion of noradrenaline* (FRIEND *et al* 1958) *Consequently most nor*adrenaline and adrenaline is excreted as different metabolites in spite of the inhibition of monoamine oxidase

Studies with Radioactive Noradrenaline and Adrenaline

SCHAYER and his co workers when using radioactive adrenaline (1951—1953) and noradrenaline (1955) found altogether five different metabolic products of adrenaline in the paper chromatography of rat urine Almost half of the adrenaline given to the rats was inactivated through losing the carbon atom of the methyl group It was concluded that this part is deaminated by monoamine oxidase (SCHAYER *et al* 1952 1953)

After β C^{14} dl noradrenaline injection the chromatography of rat urine revealed three metabolites The formation of one metabolite was prevented by iproniazid (SCHAYER *et al* 1955) Thus *monoamine* oxidase was of importance even in the metabolism of noradrenaline

In human urine 65 % of the metabolites of infused β C^{14} adrenaline lost the methyl group This suggests oxidative deamination in man (RESNICK *et al* 1958) During iproniazid treatment it has been observed that twice the amount of infused adrenaline *retains* the methyl group of the side-chain as compared with the excretion of *patients* that were not under the treatment Part of noradrenaline and adrenaline is however methylated before the oxidative deamination (AXELROD 1957) The inhibition of the monoamine oxidase activity by iproniazid results in the decrease of the 3 methoxy-4 hydroxymandelic acid excretion in man (GOODALL *et al* 1958 VON STUDNITZ 1959 KIRSHNER *et al* 1959 ZIEGLER 1960) and in test animals (AXELROD *et al* 1958 a AXELROD 1959 a KIRSHNER 1960) A corresponding increase in the excretion of the methoxy noradrenaline and methoxyadrenaline is also brought about (AXELROD 1957 GOODALL *et al* 1958) The inhibition of catechol O methyltransferase by pyrogallol similarly leads to an *increase* in the excretion of the deaminated metabolites 3 4-dihydroxymandelic acid and 3 4-dihydroxyphenylglycol after the administration of adrenaline (KIRSHNER 1960 KOPIN & AXELROD 1960) A clear decrease is produced simultaneously in the excretion of methylated metabolites Thus the metabolic pathway of noradrenaline and adrenaline can obviously be changed in case one of the enzymes is being inhibited

adrenaline and adrenaline (BURN 1952) less than ephedrine does. Consequently it has been suggested that the supersensitivity to cocaine and like compounds should not be due to monoamine oxidase inhibition (PURCHGOTT 1955). α Cocaine, a structural isomer of cocaine, inhibits monoamine oxidase as effectively as cocaine *in vitro*, but is unable to potentiate the reactions of adrenaline (FOSTER *et al* 1955). The cocaine induced supersensitivity is connected with the adrenaline content of blood (TRENDELENBURG 1959). It may be due to the changes taking place in the uptake release mechanism of noradrenaline in tissues (BEJRABLAYA *et al* 1958, WHITBY *et al* 1960, MUSCHOLL 1960, LINDMAR & MUSCHOLL 1961, HERTTING *et al* 1961).

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that even a deaminated form could be methylated in the organism 3 Methoxy 4 hydroxyphenylacetic acid was demonstrated in the urine after an oral administration of dopa (DeEds *et al* 1957 Pellerin & D'Iorio 1957) This supported the probability of O methylation under natural conditions

Axelrod (1957) found that noradrenaline and adrenaline were methylated in rat liver in the presence of S adenosylmethionine and Mg^{++} The supernatant fractions of the brain spleen and kidney homogenates can also O methylate adrenaline but in a smaller extent than the liver In rat the urinary excretion of methoxyadrenaline and 3 methoxy 4 hydroxymandelic acid after the injection of methoxyadrenaline was almost equal to that found after the administration of adrenaline (Axelrod *et al* 1958 a) This indicated that the methylated form could be further oxidized in the organism through deamination After pretreating rats with iproniazid almost all of the adrenaline given was excreted either as free or as conjugated methoxyadrenaline (Axelrod 1959 a) The excretion of 3 methoxy-4 hydroxymandelic acid decreased considerably at the same time On the basis of these findings it was concluded that monoamine oxidase deaminates primarily methylated adrenaline O methylation has been considered the main enzymatic process inactivating noradrenaline and adrenaline (Axelrod 1959 a)

Methoxynoradrenaline and methoxyadrenaline occur in human urine even under normal conditions (Axelrod *et al* 1958 b La Brosse *et al* 1958 b Goodall *et al* 1958 1959) After an intravenous administration of β H^3 adrenaline in man 90 % of the total radioactivity was excreted into the urine 45 % of this was free or conjugated methoxyadrenaline, and 30 % 3 methoxy 4 hydroxymandelic acid (La Brosse *et al* 1958 a) A similar finding was also made by Kirshner *et al* (1959)

The blood pressure response produced by methoxynoradrenaline — i e the methylated metabolite of noradrenaline — is about one 500th of that of noradrenaline (Evarts *et al* 1958) Injecting methoxynoradrenaline or methoxyadrenaline did not cause any observable physiological or psychological effects in man (Evarts *et al* 1958 La Brosse *et al* 1958 a) Bacq (1959) observed that in an anesthetized cat intravenously administered methoxynoradrenaline and methoxyadrenaline had very slight effects on vasoconstriction or on the nictitating membrane directly but they potentiated the nictitating membrane response to noradrenaline adrenaline or electric stimulation considerably Methoxynoradrenaline and adrenaline present in normal organism may thus have a physiological function in regulating the sensitivity of tissues to adrenergic transmitters Methoxyadrenaline sensitizes the nictitating membrane towards methoxyadrenaline itself (Bacq 1960) Doses of cocaine which potentiate the reactions of noradrenaline and adrenaline in the atrial preparate also potentiate the

The Physiological Significance of Monoamine Oxidase

In spite of the importance lately attached to the O-methylation of noradrenaline and adrenaline *in vivo* in some species (AXELROD 1957; ARMSTRONG *et al.* 1957) it has also been demonstrated that monoamine oxidase as well is involved in the metabolism of these hormones. This is indicated e.g. by the increase in the catecholamine content of heart muscle after the administration of iproniazid (PEKKARINEN *et al.* 1958; PLETSCHER 1958) or of harmaline and nialamide (MUSCHOLL 1959). A corresponding increase also takes place in the brain after the injection of several monoamine oxidase inhibitors of the hydrazine or nonhydrazine type (SHORE *et al.* 1957; CARLSSON *et al.* 1959 a; MUSCHOLL 1959; PLETSCHER & BESENDORF 1959; GREEN & ERICKSON 1960; FINGER 1960; EHRINGER *et al.* 1960; EVERETT & WIEGAND 1961) and in the spleen, liver, small intestine and kidney (PEKKARINEN *et al.* 1958) in 10 hours after the injection of iproniazid, but not, however, when the administration of iproniazid was continued 66 hours after the beginning of the experiment. The increase in the noradrenaline content in rat brain produced by tranylcypromine was turned into a decrease while the inhibition of monoamine oxidase was still complete (GREEN & ERICKSON 1960). It has been suggested that some other enzyme system should be responsible for the metabolism in connection with monoamine oxidase inhibition. The plasma adrenaline content is increased by harmaline and iproniazid (EAKINS & LOCKETT 1961) and the noradrenaline content of sympathetic ganglions by α-methylphenethylhydrazine (SPECTOR *et al.* 1960 a). The monoamine oxidase inhibitors propamidine, ephedrine, *metamphetamine and cholin-p-tolylether do not, however, affect* the noradrenaline content of cat spleen, heart or liver (VON EULER & HELLNER-BJORKMAN 1955). It has been supposed that the main function of monoamine oxidase under physiological conditions, at least in the brain, is the intracellular regulation of the noradrenaline and serotonin contents in the neurons, so that the constant access of these amines to the receptors is interrupted (SPECTOR *et al.* 1960 b). The noradrenaline content in the brain under normal conditions may be influenced by the activity of monoamine oxidase on dopamine, which is a particularly good substrate.

D. *Catechol-O-methyltransferase*

The urinary excretion of a metabolite of noradrenaline and adrenaline, 3-methoxy-4-hydroxymandelic acid, was increased on the parenteral administration of noradrenaline and adrenaline and on the oral administration of 3,4-dihydroxymandelic acid (ARMSTRONG *et al.* 1957). This shows

that even a deaminated form could be methylated in the organism 3-Methoxy-4-hydroxyphenylacetic acid was demonstrated in the urine after an oral administration of dopa (DeEds *et al* 1957 Pellerin & Di Iorio 1957) This supported the probability of O methylation under natural conditions.

Axelrod (1957) found that noradrenaline and adrenaline were methylated in rat liver in the presence of S adenosylmethionine and Mg^{++} The supernatant fractions of the brain, spleen and kidney homogenates can also O methylate adrenaline but in a smaller extent than the liver In rat the urinary excretion of methoxyadrenaline and 3 methoxy-4 hydroxymandelic acid after the injection of methoxyadrenaline was almost equal to that found after the administration of adrenaline (Axelrod *et al* 1958 a) This indicated that the methylated form could be further oxidized in the organism through deamination After pretreating rats with iproniazid almost all of the adrenaline given was excreted either as free or as conjugated methoxyadrenaline (Axelrod 1959 a) The excretion of 3 methoxy-4 hydroxymandelic acid decreased considerably at the same time On the basis of these findings it was concluded that monoamine oxidase deaminates primarily methylated adrenaline O methylation has been considered the main enzymatic process inactivating noradrenaline and adrenaline (Axelrod 1959 a)

Methoxynoradrenaline and methoxyadrenaline occur in human urine even under normal conditions (Axelrod *et al* 1958 b La Brosse *et al* 1958 b Goodall *et al* 1958 1959) After an intravenous administration of β H^3 adrenaline in man 90 % of the total radioactivity was excreted into the urine 45 % of this was free or conjugated methoxyadrenaline, and 30 % 3 methoxy-4 hydroxymandelic acid (La Brosse *et al* 1958 a) A similar finding was also made by Kirshner *et al* (1959)

The blood pressure response produced by methoxynoradrenaline — i e the methylated metabolite of noradrenaline — is about one 500th of that of noradrenaline (Evarts *et al* 1958) Injecting methoxynoradrenaline or methoxyadrenaline did not cause any observable physiological or psychological effects in man (Evarts *et al* 1958 La Brosse *et al* 1958 a) Bacq (1959) observed that in an anesthetized cat intravenously administered methoxynoradrenaline and methoxyadrenaline had very slight effects on vasoconstriction or on the nictitating membrane directly but they potentiated the nictitating membrane response to noradrenaline adrenaline or electric stimulation considerably Methoxynoradrenaline and adrenaline, present in normal organism may thus have a physiological function in regulating the sensitivity of tissues to adrenergic transmitters. Methoxyadrenaline sensitizes the nictitating membrane towards methoxyadrenaline itself (Bacq 1960) Doses of cocaine which potentiate the reactions of noradrenaline and adrenaline in the atrial preparate, also *potentiate the*

The Physiological Significance of Monoamine Oxidase

In spite of the importance lately attached to the O-methylation of noradrenaline and adrenaline *in vivo* in some species (AXELROD 1957, ARMSTRONG *et al.* 1957) it has also been demonstrated that monoamine oxidase as well is involved in the metabolism of these hormones. This is indicated e.g. by the increase in the catecholamine content of heart muscle after the administration of iproniazid (PEKKARINEN *et al.* 1958, PLETSCHER 1958) or of harmaline and malamide (MUSCHOLL 1959). A corresponding increase also takes place in the brain after the injection of several monoamine oxidase inhibitors of the hydrazine or nonhydrazine type (SHORE *et al.* 1957, CARLSSON *et al.* 1959 a, MUSCHOLL 1959, PLETSCHER & BESENDORF 1959, GREEN & ERICKSON 1960, FINGER 1960, FUHRINGER *et al.* 1960, EVERETT & WIEGAND 1961) and in the spleen, liver, small intestine and kidney (PEKKARINEN *et al.* 1958) in 10 hours after the injection of iproniazid but not, however, when the administration of iproniazid was continued 66 hours after the beginning of the experiment. The increase in the noradrenaline content in rat brain produced by tranylcypromine was turned into a decrease while the inhibition of monoamine oxidase was still complete (GREEN & ERICKSON 1960). It has been suggested that some other enzyme system should be responsible for the metabolism in connection with monoamine oxidase inhibition. The plasma adrenaline content is increased by harmaline and iproniazid (EAKINS & LOCKETT 1961) and the noradrenaline content of sympathetic ganglions by α-methylphenethylhydrazine (SPECTOR *et al.* 1960 a). The monoamine oxidase inhibitors propamidine, ephedrine, metamphetamine and cholin *p*-tolylether do not, however, affect the noradrenaline content of cat spleen, heart or liver (VON EULER & HELLNER-BJORKMAN 1955). It has been supposed that the main function of monoamine oxidase under physiological conditions, at least in the brain, is the intracellular regulation of the noradrenaline and serotonin contents in the neurons so that the constant access of these amines to the receptors is interrupted (SPECTOR *et al.* 1960 b). The noradrenaline content in the brain under normal conditions may be influenced by the activity of monoamine oxidase on dopamine, which is a particularly good substrate.

D. *Catechol-O-methyltransferase*

The urinary excretion of a metabolite of noradrenaline and adrenaline, 3-methoxy-4-hydroxymandelic acid, was increased on the parenteral administration of noradrenaline and adrenaline and on the oral administration of 3,4-dihydroxymandelic acid (ARMSTRONG *et al.* 1957). This shows

animals and tissues and is localized in the soluble fraction of cells (AXELROD *et al* 1959 a AXELROD & TOMCHICK 1958 AXELROD 1959 b) The strongest enzyme activity has been observed in the liver Large amounts of catechol O methyltransferase also appear in glandular tissues e g in the salivary glands pancreas and adenohypophysis Catechol O methyltransferase has also been found in all peripheral nerves whereas it has not been observed in the skeletal muscle and only in negligible amounts in the heart muscle Animal species differ in this respect (SHIDEMAN & GOLDBERG 1961) Clear catechol-O methyltransferase activity occurs in the brain of the monkey, but only traces of it are observed in the brain of the rat and the cat (AXELROD *et al* 1959 c) Catechol O methyltransferase and monoamine oxidase differ probably in their physiologic functions so that catechol O methyltransferase inactivates the noradrenaline and adrenaline present in the circulation whereas monoamine oxidase metabolizes catecholamines in tissues (SPECTOR *et al* 1960 a 1960 b) Following radioactive adrenaline infusions methoxyadrenaline was observed to be the largest fraction (28%) in cat tissue (KIRSHNER 1960) Still 3 4-dihydroxymandelic acid and 3 4 dihydroxyphenylglycol were also found in tissues indicating that a direct deamination of part of the infused adrenaline also takes place in them by monoamine oxidase In the liver the largest fraction was 3 4 dihydroxymandelic acid in the small intestine it was 3 4-dihydroxyphenylethylglycol and 3 methoxy-4 hydroxyphenylethylglycol

Inhibitors of Catechol O methyltransferase

Pyrogallol and catechol potentiate the nictitating membrane and vasopressor responses to some extent (BACQ 1936 WYLIE 1961, WYLIE *et al* 1960) but probably only following an administration of cocaine (LEMBECK & RESCH 1960) Pyrogallol and the other catechols *prevent competitively O methylation in vitro* Pyrogallol retarded H^3 adrenaline metabolism in the whole rat Compounds lengthening the half life of noradrenaline are e g pyrogallol glycocyamine 3 4 5 trihydroxyphenylethylamine catechol quercetine and compounds of tropoloneacetamide series (UDENFRIEND *et al* 1959 AXELROD & TOMCHICK 1959 CARLSSON *et al* 1962) Though the formation of methoxynoradrenaline can be prevented almost completely the half life of noradrenaline cannot be affected to such an extent (UDENFRIEND *et al* 1959) If catechol O methyltransferase is inhibited there must exist other alternative pathways for noradrenaline metabolism Pyrogallol does not produce any increase in the noradrenaline content of rat brain or heart (CROUT *et al* 1960) and it does not inhibit the decrease in the brain noradrenaline content brought about by reserpine (SPECTOR *et al* 1960 b) In addition it has been shown more recently that the increase

positive chrono and inotropic effects of the 3 methoxy catechols (HOLTZ *et al* 1960) The vasopressor and nictitating membrane responses to the O methylated metabolites of noradrenaline and adrenaline were *not observed* — contradictory to the finding by BACQ — to be potentiated by a monoamine oxidase inhibitor (HOLTZ *et al* 1960) The potentiation, however, occurred in connection with the methylated *products of tyramine*

Using partially purified enzyme preparations in the study of the metabolism of noradrenaline, adrenaline and their O methyl analogs (LEEPER *et al* 1958) it was seen that O *methylation converted noradrenaline* to a substance which was more sensitive to monoamine oxidase than noradrenaline Noradrenaline and adrenaline as well as their acid end products also functioned as the substrates of catechol O methyltransferase *in vitro* According to a more recent contradictory opinion the methylated compounds are not decomposed by monoamine oxidase more rapidly than primary noradrenaline and adrenaline (HOLTZ *et al* 1960) *According to* HOLTZ and others noradrenaline and adrenaline as well as the methylated forms are deaminated through oxidation which occurs at half the rate involved in the oxidation of tyramine This has been demonstrated with mitochondrial preparates from the liver, brain and kidney of different animal species by using these substances as substrates

The presence of methoxynoradrenaline was demonstrated chromatographically in the brains of rats treated with iproniazid (AXELROD 1958) No methoxynoradrenaline was observed in the brains of untreated rats *In vitro* incubation of noradrenaline in rat brain also produced methoxy noradrenaline Methoxynoradrenaline and methoxyadrenaline have been observed to occur in the adrenal glands and spleen in normal conditions (AXELROD *et al* 1958 b) After an administration of labelled adrenaline it was discovered in the heart spleen and several glandular tissues e g salivary gland pancreas and the adrenal gland but only in a small extent in the skeletal muscle and not at all in the brain of the cat (AXELROD *et al* 1959 b, 1959 c) As soon as two minutes after the injection, the concentration of methoxyadrenaline was higher than that of adrenaline in most tissues, which indicated a rapid O methylation in the organism The methoxy adrenaline content was great in the liver, heart, kidney, pancreas and lung but very small in the brain The ratio between the monoamine oxidase and catechol O methyltransferase activities in the rat brain and heart *in vitro* was 25—50 times that seen in the liver (CROUT *et al* 1960) The disappearance of labelled adrenaline from plasma takes place in two phases 1) a rapid decrease which presents the diffusion into tissues and O methylation, 2) a slower release from tissues and competitive metabolism (AXELROD *et al* 1959 c)

Catechol O methyltransferase has an extensive occurrence in different

animals and tissues and is localized in the soluble fraction of cells (AXELROD *et al.* 1959 a; AXELROD & TOMCHICK 19[illegible]; AXELROD 19[illegible] b). The strongest enzyme activity has been observed in the liver. Large amounts of catechol-O-methyltransferase also appear in glandular tissues, e.g. in the salivary glands, pancreas and adenohypophysis. Catechol-O-methyltransferase has also been found in all peripheral nerves, whereas it has not been observed in the skeletal muscle and only in negligible amounts in the heart muscle. Animal species differ in this respect (SHIDEMAN & GOLDBERG 19[illegible]). Clear catechol-O-methyltransferase activity occurs in the brain of the monkey but only traces of it are observed in the brain of the rat and the cat (AXELROD *et al.* 1959 c). Catechol-O-methyltransferase and monoamine oxidase differ probably in their physiologic functions so that catechol-O-methyltransferase inactivates the noradrenaline and adrenaline present in the circulation whereas monoamine oxidase metabolizes catecholamines in tissues (SPECTOR *et al.* 1960 a, 1960 b). Following radioactive adrenaline infusions metanephrine adrenaline was observed to be the largest fraction (25%) in cat tissue (KIRSHNER 1960). Small 3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylglycol were also found in tissues indicating that a direct deamination of part of the infused adrenaline also takes place in them by monoamine oxidase. In the liver the largest fraction was 3,4-dihydroxymandelic acid, in the small intestine it was 3,4-dihydroxyphenylethyleneglycol and 3-methoxy-4-hydroxyphenylethyleneglycol.

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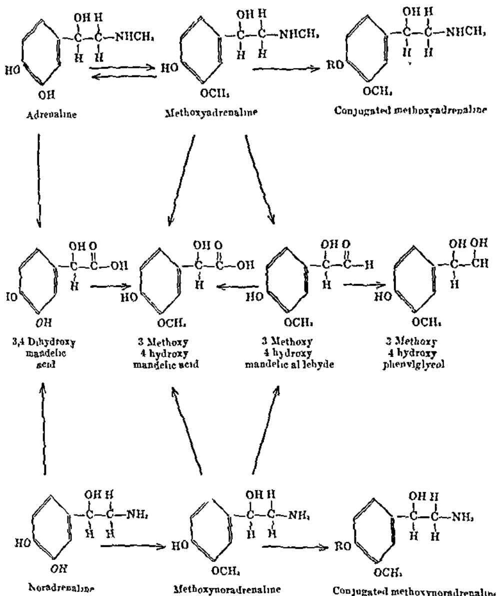


Figure 1 Routes of metabolism of noradrenaline and adrenaline according to Axelrod (1960)

in the tissue noradrenaline content produced by monoamine oxidase inhibitors is prevented by simultaneous treatment with certain inhibitors of catechol O methyltransferase (CARLSSON *et al* 1962)

Summary of Earlier Studies

On the basis of present knowledge two enzymatic inactivation pathways participate in the metabolism of noradrenaline and adrenaline the oxidative deamination of the amino group of the side chain by monoamine oxidase and the methylation of the phenolic hydroxyl group by catechol O methyltransferase The excretion of free or conjugated adrenaline comprises only a small part of the elimination process The oxidation of noradrenaline and adrenaline by cytochrome oxidase has not been clarified *in vivo* conditions The relative parts played by monoamine oxidase and catechol O methyltransferase in the inactivation of noradrenaline and adrenaline in the organism still remain open Injecting simultaneously H^3 adrenaline and methoxyadrenaline in which the methoxy group was labelled with C^{14} and determining the ratios of the isotopes found in each of the metabolites in the urine (KOPIN 1960) it was calculated that about 70 % of the adrenaline given was O methylated to methoxyadrenaline of which 25 % was further deaminated to 3 methoxy 4 hydroxymandelic acid and 3 methoxy 4 hydroxyphenylglycol Since 45 % of adrenaline was excreted as 3 methoxy 4 hydroxymandelic acid about 20 % must have been formed primarily through deamination About 68 % of the adrenaline given was concluded to be O methylated and 23 % to be deaminated oxidized or reduced The rest is excreted unchanged and in a conjugated form Most noradrenaline and adrenaline injected undergoes O methylation primarily but different conditions may be involved in the metabolism of the endogenous noradrenaline in tissues

*In rat brain and heart the main metabolism of endogenous nor*adrenaline occurs through oxidative deamination (CROUT *et al* 1961) However the tritium labelled noradrenaline taken up by cat spleen and thereafter released through electric stimulation is mainly O methylated (HERTTING & AXELROD 1961)

In spite of the fact that there are several strong inhibitors affecting monoamine oxidase and catechol O methyltransferase *in vitro* the biological reactions of noradrenaline and adrenaline after administration of *these inhibitors in vivo undergo quite small changes and the excretion of* these substances into the urine in pure forms is very small Thus the biological inactivation of noradrenaline and adrenaline may still include other factors affecting the metabolism

The routes of the metabolism of noradrenaline and adrenaline *according* to AXELROD (1960) have been shown in figure 1

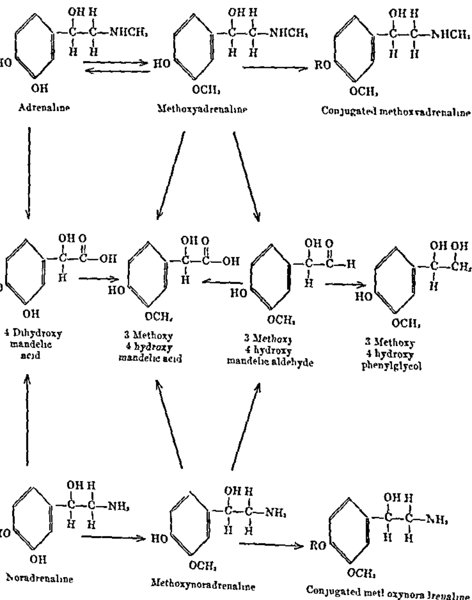


Figure 1 Routes of metabolism of noradrenaline and adrenaline according to Axelrod (1960)

OWN INVESTIGATIONS

The Object of the Present Study

At the beginning of this work most studies *in vitro* concerning the metabolism of noradrenaline and adrenaline were done without using the amines themselves as substrates. The tissues contain quite large amounts of endogenous noradrenaline when compared e.g. to blood or urine. Consequently it was considered important to study the tissue metabolism of noradrenaline and adrenaline. The basic principles of the method have been published previously (Iisalo & Pekkarinen 1958). The role played by monoamine oxidase enzyme in this metabolism was especially the object of the present investigation. This enzyme has, for a long time, been considered the principal inactivator of noradrenaline and adrenaline in the organism. The purpose of the study is to answer the following questions.

1. Can the monoamine oxidase in bovine tissues metabolize noradrenaline and adrenaline *in vitro* and is it possible to prevent the metabolism with specific enzyme inhibitors?

2. Is there any indication of the existence of cytochrome oxidase or any other heavy metal catalysis metabolizing noradrenaline and adrenaline in bovine tissues?

3. Does the enzymatic metabolism of noradrenaline in small endogenous tissue contents or in concentrations near to biological conditions take place in the same way as the metabolism of large added quantities of noradrenaline and adrenaline? Do the enzyme inhibitors prevent the metabolism of noradrenaline in these concentrations in the same way as in high concentrations?

4. Do the monoamine oxidase inhibitors used in this study when injected *in vivo* decrease the enzymatic activity of tissues metabolizing noradrenaline and adrenaline?

ENZYMATIC METABOLISM OF NORADRENALINE AND ADRENALINE IN BOVINE TISSUES *IN VITRO*

1. Chemical Determination

A Method for Incubation and Chemical Determination

In this study noradrenaline and adrenaline were used as specific substrates when studying the enzymatic activity of tissue homogenates (IISALO & PEKKARINEN 1954, 1958) After incubation the noradrenaline and adrenaline not metabolized were extracted from the homogenate with trichloroacetic acid The acid was extracted from the filtrate with ether Noradrenaline and adrenaline were determined spectrophotometrically at pH 6 by the iodine oxidation reaction as noradreno- and adrenochrome (VON EULER & HAMBERG 1949) Excess iodine was removed with sodium thiosulphate

Red noradreno or adrenochrome are not usually produced by the enzymatic metabolism of noradrenaline and adrenaline in the animal organism The method is convenient for studying the enzymatic activity of tissues by means of determining the unmetabolized noradrenaline and adrenaline The amount of metabolized noradrenaline and adrenaline represents the enzymatic activity The method being used for preparing the samples has been described previously (IISALO & PEKKARINEN 1958) 50 g of fresh bovine tissue was minced (Top Drive Macerator, Townson & Mercer Ltd or DGM homogenisator Edmund Buhler, Tubingen) in 0 2 M Na_2HPO_4 and KH_2PO_4 buffer at pH 7 4 for 1 5—3 minutes To decrease autoxidation defibrinated pig blood was used instead of phosphate as buffer after the first reported experiments with heart muscle 100 g of tissue was minced in 250 ml of distilled water 67 ml of defibrinated pig blood was then added to 100 ml of this suspension after which the volume was added up to 200 ml with distilled water 5 ml of the suspension was dispensed into 100 ml test tubes The enzyme source the bovine tissue always comprised 10 % (w/v) of the suspension The final suspension for incubation (10 ml) contained about 1 6 ml of blood The enzymatic activity was preserved although the tissue had been stored in a refrigerator (—15°C) for 24 hours However, most experiments were made using fresh bovine tissues

Enzyme inhibitors were added to the 5 ml tissue homogenate in the

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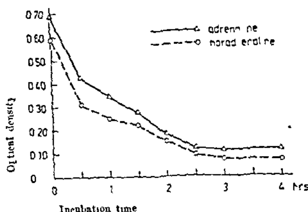


Figure 2

The metabolism of noradrenaline (N) and adrenaline (A) in a bovine liver homogenate

The optical density due to noradreno- and adrenochrome during incubation at 37°C 5.92 µmol of N and 5.46 µmol of A were added to 10 ml of 10 % (w/v) homogenate The determination of the red colour was made after oxidation of the eluate with iodine in Beckman B standard test tube cuvettes

3,4-dihydroxymandelic acid which gives a similar yellowish colour in iodine oxidation at pH 6 (cf page 34) In some cases the intensity of the pigment increased when incubation was continued When incubating without noradrenaline and adrenaline the optical density of the tissue blank was 0.01—0.02 at 529 mµ The formation of the yellowish pigment in these samples was negligible Consequently the pigment was largely due to the catechol amines Due to this methodic feature the measurable amount of noradrenaline and adrenaline is not reduced to zero even at the minimum there appears to be yellow colour with a 5—20 % intensity (reading 0.04—0.12) of the original optical density In calculations and in evaluating the inhibition produced by enzyme inhibitors the enzyme activity in each series has been calculated as 100 % and the noradrenaline and adrenaline that have remained unmetabolized (or the colour observed after the incubation) have not been regarded in the results Thus the difference between the photometric reading taken after the incubation without enzyme inhibitors and that taken before the incubation indicates the *maximum metabolism under experimental conditions, i e* it represents the *enzyme activity* The enzyme activity has been marked 100 % and its inhibition at different inhibitor concentrations has been given in per cent

The colour produced in the iodine oxidation reaction by some of the known metabolic products of noradrenaline and adrenaline was studied at pH 6 and 529 mµ The added amounts of these substances were equimolar as regards the concentrations of noradrenaline and adrenaline The

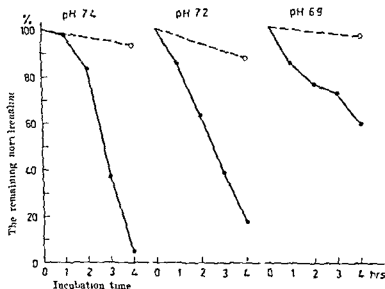
test tubes. As a substrate 1 ml (1 mg) of 0,1 % l-adrenaline (Merck) or l-noradrenaline bitartrate (Winthrop or Boehringer, calculated as base) were added. The final volume of the suspension thus contained 5.92 μmol of noradrenaline or 5.46 μmol of adrenaline. The pH of the suspension was adjusted to 7.30—7.45 using 0.2 M pH 7.5 phosphate buffer or 0.25 N NaOH. In 100 ml test tubes the final volume of the samples was then made up to 10 ml with distilled water. The test tubes were closed with rubber stoppers through which an oxygen stream was led using glass tubes that reached down to the bottom of the test tubes. The oxygen was removed using another glass tube. The test tubes were connected into a series, a maximum of eight tubes belonging to one series. The stream from the oxygen tank into the tubes could be regulated by clamps. It was the same for all the series. The continuous oxygen stream kept the tissue homogenate in a bubbling motion. The incubation was carried out at 37°C during 1—4 hours in a small room equipped with a thermostat.

After the incubation the proteins were precipitated with 5 ml of 10 % trichloroacetic acid. The samples were centrifuged or filtered either 15 min later or on the following day. Trichloroacetic acid was extracted three times with an equal volume of ether (IISALO & PEKKARINEN 1958). The pH of the eluate was then increased up to 6.0 by adding 2 ml of 2 N sodium acetate-acetic acid buffer (pH 6.3) to 4 ml of the filtrate.

The determination of large amounts of noradrenaline and adrenaline was performed using the iodine oxidation method of VON EULER and HAMBERG (1949). After a 3 minute oxidation at pH 6 with 0.5 ml of 0.1 N iodine the remaining iodine was removed by adding 0.6 ml of 0.1 N sodium thiosulphate. The noradreno- and adrenochrome were determined photometrically using standard test tube cuvettes in a Beckman B spectrophotometer at 529 mμ and distilled water as a control.

The speed of noradrenaline and adrenaline metabolism varied greatly from tissue to tissue (see table 6, page 38) and even in the same tissues taken from different animals. Consequently the incubation time could not be kept the same in all the tests. Thus unmetabolized noradrenaline and adrenaline were left e.g. in heart muscle tissue in most cases after 4 hours of incubation, whereas noradrenaline and adrenaline in a liver tissue (fig. 2) were metabolized within 2½ hours. If incubation were continued at this point up to 4 hours even the noradrenaline and adrenaline which had not been metabolized in the presence of enzyme inhibitors would be destroyed primarily through autoxidation. It can also be seen in the figure that in the determination a small amount of yellowish colour remains in the suspension. This is not due to noradreno- or adrenochrome. Yellow-brownish pigment was formed in all the tissues when incubated with noradrenaline and adrenaline. The pigment was not analysed but it might be due to

NORADRENALINE



ADRENALINE

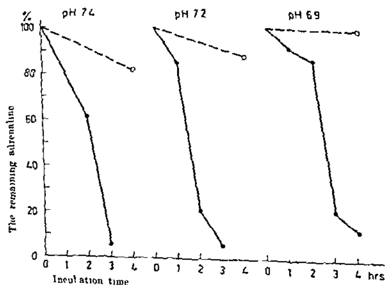


Figure 5

Table 4

The rate of metabolism at different substrate concentrations

Noradrenaline (N) and adrenaline (A) have been added to heart muscle homogenate and incubated one hour at 37°C

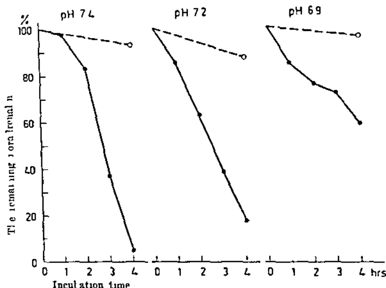
Added µmol N	Metabolized µmol N	Added µmol A	Metabolized µmol A
1.48	0.8	[illegible]	0.8
2.96	1.4	2.73	1.2
5.92	1.8	5.46	1.9
11.84	1.8	10.92	1.9
23.68	1.9	21.84	2.0

optical density readings as compared with the water blank were as follows methoxynoradrenaline 0.020, methoxyadrenaline 0.025, 3-methoxy-4-hydroxymandelic acid 0.0 and 3,4-dihydroxymandelic acid 0.110

The saturation of the enzyme with a substrate was studied adding noradrenaline and adrenaline to the heart muscle suspension as substrates The different samples contained 0.25, 0.5, 1.0, 2.0 and 4.0 mg of noradrenaline or adrenaline The samples were incubated for one hour Each substrate concentration was represented by two simultaneous samples Table 4 shows the rate of metabolism at various concentrations The rate of noradrenaline and adrenaline metabolism did not increase when the concentrations were made higher than those used in the studies i.e. 592 µM noradrenaline and 546 µM adrenaline The enzyme can thus be considered saturated with the substrate in the present studies

Small amounts of noradrenaline and adrenaline may have been destroyed through autoxidation during the aerobic incubation of tissue samples (IISALO & PEKKARINEN 1958) However the tissues are normally good stabilisators for noradrenaline and adrenaline To estimate the amount of autoxidation most series included a control sample where the tissue suspension had been heated before the addition of the substrate in order to destroy the enzymatic activity The autoxidation of this heated control sample is however somewhat greater than in the normal tissue homogenate since the colloidal form of the normal tissues which protects them from oxidation, is destroyed by heating and the oxidation of noradrenaline and adrenaline is increased Autoxidation unavoidable in the earlier experiments (IISALO & PEKKARINEN 1958) could be somewhat decreased by using defibrinated blood to buffer the tissue homogenates Adjusting the pH at 7.4 it was tried to avoid autoxidation which appears at a higher pH Figure 3 represents the effect of pH on the rate of noradrenaline and adrenaline metabolism and on the amount of autoxidation determined in the heart muscle suspension Since the amount of autoxidation cannot be

NORADRENALINE



ADRENALINE

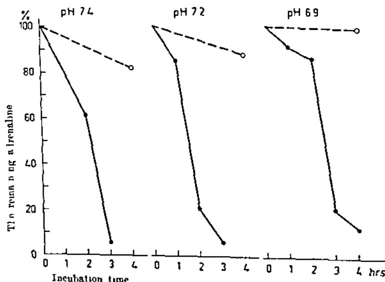


Figure 3

The effect of pH on the metabolic rate and on the autoxidation amount of noradrenaline and adrenaline in 10 % (w/v) heart muscle homogenate buffered with swine blood (about 1.6 ml/10 ml). Continuous line = enzymatic metabolism, broken line = autoxidation on heat destruction inactivated samples

Table 4

The rate of metabolism at different substrate concentrations

Noradrenaline (N) and adrenaline (A) have been added to heart muscle homogenate and incubated one hour at 37°C

Added µmol N	Metabolized µmol N	Added µmol A	Metabolized µmol A
1.18	0.8	1.39	0.8
[illegible]	1.4	2.73	1
5.9	1.8	5.46	1.9
11.84	1.8	10.92	1.9
[illegible]	1.9	21.84	2.0

optical density readings as compared with the water blank were as follows: methoxynoradrenaline 0.020, methoxyadrenaline 0.025, 3-methoxy-4-hydroxymandelic acid 0.0, and 3,4-dihydroxymandelic acid 0.110.

The saturation of the enzyme with a substrate was studied adding noradrenaline and adrenaline to the heart muscle suspension as substrates. The different samples contained 0.25, 0.5, 1.0, 2.0 and 4.0 mg of noradrenaline or adrenaline. The samples were incubated for one hour. Each substrate concentration was represented by two simultaneous samples. Table 4 shows the rate of metabolism at various concentrations. The rate of noradrenaline and adrenaline metabolism did not increase when the concentrations were made higher than those used in the studies, i.e. 592 µM noradrenaline and 546 µM adrenaline. The enzyme can thus be considered saturated with the substrate in the present studies.

Small amounts of noradrenaline and adrenaline may have been destroyed through autoxidation during the aerobic incubation of tissue samples (IISALO & PEKKARINEN 1958). However, the tissues are normally good stabilisators for noradrenaline and adrenaline. To estimate the amount of autoxidation most series included a control sample where the tissue suspension had been heated before the addition of the substrate in order *to destroy the enzymatic activity*. The autoxidation of this heated control sample is, however, somewhat greater than in the normal tissue homogenate, since the colloidal form of the normal tissues, which protects them from oxidation, is destroyed by heating, and the oxidation of noradrenaline and adrenaline is increased. Autoxidation unavoidable in the earlier experiments (IISALO & PEKKARINEN 1958) could be somewhat decreased by using defibrinated blood to buffer the tissue homogenates. Adjusting the pH at 7.4 it was tried to avoid autoxidation which appears at a higher pH. Figure 3 represents the effect of pH on the rate of noradrenaline and adrenaline metabolism and on the amount of autoxidation determined in the heart muscle suspension. Since the amount of autoxidation cannot b

samples were preincubated for half an hour with inhibitors before adding noradrenaline. Thus the temperature remained exactly constant for the whole time.

Adding increasing amounts of noradrenaline and adrenaline to an ordinary liver tissue suspension it was possible to draw a calibration curve for the optical density values. It was a straight line as expected (fig. 4). The average of optical density value (Beckman B) corresponding to 1 mg was 0.59 (s.e.m. ±0.011) for noradrenaline and 0.69 (s.e.m. ±0.024) for adrenaline at wave length 529 mμ in test tube cuvettes. The optical density corresponding to the other concentrations can be seen in the figure.

To find out the s.e.m. of one series of experiments, a liver suspension was prepared in the usual way and then incubated with noradrenaline and adrenaline. Incubation lasted for 3 hours and was performed both without enzyme inhibitors and with iproniazid and amphetamine. Five simultaneous *determinations* were done in each series and the remaining noradrenaline and adrenaline were assayed in the usual way. Table 5 shows the results of these experiments.

Table 5

The standard error of the mean (s.e.m.) and the standard deviation (s) of one series of experiments. (Iproniazid given as phosphate, amphetamine as sulphate)

Substrate (0.1 mg/ml)	Enzyme inhibitor mg/ml		Number of tests	Remaining substrate (in %)	s.e.m.	s
Noradrenaline	—		5	14	±0.4	±1.0
	iproniazid	0.2	5	41	±2.5	±5.7
	amphetamine	1.0	5	73	±0.6	±1.4
Adrenaline	—		5	15	±0.6	±1.4
	iproniazid	0.2	5	61	±1.5	±3.4
,	amphetamine	1.0	5	79	±0.4	±1.0

In this work standard methods of statistical analysis were applied to the data obtained. The *difference* between the *per cent* of inhibition at one inhibitor concentration and the *zero* line was considered significant if the probability (P) was $\leqq 0.05$ and not significant with $P > 0.05$. The parallel increase in the inhibition of the *enzymatic* metabolism of noradrenaline and *adrenaline* with the increase *in* the inhibitor concentration renders the obtained results more significant.

B. Results

The initial rate of the *normal* enzymatic *metabolism* of noradrenaline and adrenaline under experimental *conditions in* different bovine tissue

expressed accurately and as it usually varied from 0 to 10 % of the metabolized amount and never exceeded 20 % this methodical error has remained in the calculations.

It has been reported earlier (IISALO & PEKKARINEN 1958) that by using an air stream instead of an oxygen stream the rate of metabolism was clearly reduced, and if no air or oxygen stream was used there occurred no metabolism at all in the heart muscle suspension during the first 4 hours. When the contact of oxygen and the suspension was improved by means of shaking the test tubes simultaneously with the oxygenation, the speed of metabolism was increased. Because of this important role played by oxygen pressure, the experiments had to be performed using an even oxygen stream to produce a similar bubbling motion simultaneously in all the test tubes belonging to the same series.

Temperature also affected the metabolism rate of noradrenaline and adrenaline clearly (IISALO & PEKKARINEN 1958). Although the experiments were carried out at 37°C, which was also the temperature of the oxygen it was still possible that the temperature of the samples taken from room temperature was not uniform during the first 15 minutes and therefore the metabolic rate of noradrenaline and adrenaline was slower than later. In experiments where only a few times higher than normal noradrenaline concentrations in tissues were used, this factor did not disturb since these

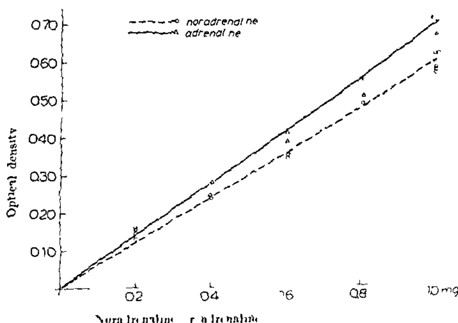


Figure 4

The calibration curves for noradrenaline and adrenaline in 10 % (w/v) liver homogenate when 0.2—1.0 mg of noradrenaline or adrenaline is added. The optical density is measured in Beckman B standard test tube cuvettes at wave length [illegible] mμ. The final volume for colorimetric determination is 5.1 ml.

1 Heart Muscle

Noradrenaline and adrenaline were metabolized almost at the same rate in bovine heart muscle under experimental conditions Still, adrenaline showed a slight tendency to slower metabolism (figs 5 and 6) In experiments with enzyme inhibitors a 4 hour incubation time was used On the average 74 ± 3 4 % (4 4 μmol) of the added 5 92 μmol (1 mg) noradrenaline and 86 ± 1 8 % (4 7 μmol) of the added 5 46 μmol (1 mg) adrenaline were metabolized during the 4 hour incubation

Enzyme Inhibitors

Iproniazid phosphate (1 isonicotinyl 2 isopropylhydrazine) and amphetamine sulphate were used as the inhibitors of the activity of the mono amine oxidase The enzymatic metabolism of noradrenaline and adrenaline in experimental conditions corresponded to the inhibitor concentration (fig 7) decreasing when the concentration was increased

e homogenates The enzyme activity at different inhibitor concentrations
r continuous oxygenation. The enzyme activity is expressed as per cent of the maximum number of single determinations (n) on which the mean values are based

Uterus		Spleen		Liver		Kidney		Brain	
4		4		1		1		1	
N	A	N	A	N	A	N	A	N	A
% n	% n	% n	% n	% n	% n	% n	% n	% n	% n
5 ([illegible])	33 ([illegible])			[illegible] (3)	[illegible] (3)	66 (3)	41 (3)		
1 (4)	40 (3)	33 (2)	36 (3)	64 (3)	63 (3)	78 (2)	[illegible] (2)	71 (3)	56 (3)
0 (4)	73 (4)	73 (3)	63 (3)					83 (3)	82 (3)
0 (2)	89 (3)	88 (3)	83 (3)	85 (3)	79 (3)	93 (3)	89 (3)	99 (3)	93 (3)
				97 (3)	97 (3)	97 (2)	97 (2)		
				24 (3)	21 (3)	14 (3)	17 (3)		
[illegible] (4)	13 (4)	[illegible] (3)	[illegible] ( )	70 (3)	37 (3)	41 (2)	20 (2)	3 ([illegible])	10 ([illegible])
3 (3)	59 (3)	[illegible] (3)	61 (3)					5 (3)	12 (3)
34 (4)	( )	5[illegible] (3)	72 (2)	[illegible] (3)	77 (3)	59 (2)	63 (2)	17 (3)	21 (3)
				9 (3)	9[illegible] (3)	94 (2)	82 (2)		
00 (1)	98 (1)	9 ([illegible])	91 (2)	9[illegible] (3)	98 (3)	93 (3)	98 (3)		
88 (4)	89 (4	94 (3)	89 ([illegible])	98 (2)	93 (3)	98 (1)	99 (2)	99 (3)	85 (3)
89 (3)	93 (3)	1[illegible] (3)	91 (2)					6 ([illegible])	93 (3)
[illegible] 3)	94 (4)	93 (2)	100 (2)	99 (3)	99 (3)	99 (2)	100 (2)	100 (3)	100 (3)
				99 (2)	99 (2)	100 (1)	100 (1)		

Table 6

The metabolized amount of noradrenaline and adrenaline during the first hour of incubation at 37°C. 5.92 μmol of noradrenaline or 5.4[illegible] μmol of adrenaline was added in 10 ml of 10 % (w/v) tissue suspension

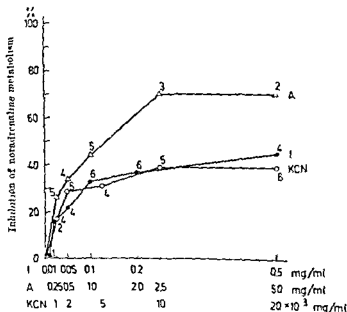
Tissue	Noradrenaline μmol (± s.e.m.)	Adrenaline μmol (± s.e.m.)
Heart muscle	0.8 ± 0.14	0.8 ± 0.14
Skeletal muscle	0.2 ± 0.09	0.3 ± 0.09
Uterine tissue	1.0 ± 0.01	0.7 ± 0.11
Intestinal wall	1.6 ± 0.25	1.6 ± 0.[illegible]
Spleen	2.3 ± 0.17	2.2 ± 0.[illegible]
Lung	3.5 ± 0.08	3.[illegible] ± 0.18
Brain	1.8 ± 0.02	1.9 ± 0.09
Kidney	4.6 ± 0.10	3.8 ± 0.10
Liver	4.3 ± 0.15	4.[illegible] ± 0.[illegible]

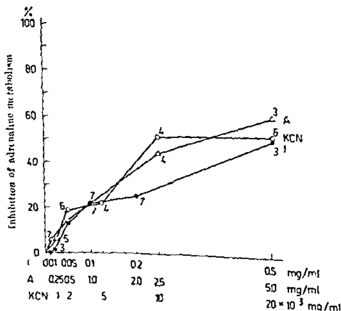
homogenates has been presented in table 6. The results from the experiments where noradrenaline and adrenaline were incubated in the presence of enzyme inhibitors have been summarized in table 7.

Table 7. The metabolism of noradrenaline (N) and adrenaline (A) in bor[illegible]
1 mg of N or A was added to 10 ml of 10 % (w/v) tissue homogenate and incubated at 3[illegible] [illegible] amount of N or A metabolized in control samples during incubation. In bracke[illegible]

Tissue		Heart		Lung		Intestin[illegible]	
Incubation time in hrs				[illegible]		4	
Enzyme inhibitor	mg/ml	N	A	N	A	N	A
		% n	% n	% n	% n	% n	% n
Iproniazid phosphate	0.5	54 (4)	50 (3)	53 ([illegible])	50 (2)		
	0.2	63 ([illegible])	[illegible] (7)	53 ([illegible])	48 ([illegible])	46 (4)	44 (4)
	0.1	[illegible] ([illegible])	[illegible] ([illegible])	70 (1)	[illegible] (1)	[illegible] (4)	[illegible] (4)
	0.05	[illegible] (1)	8[illegible] (4)	[illegible] (2)	[illegible] ([illegible])	[illegible] (4)	[illegible] (4)
	0.02	[illegible] ([illegible])	[illegible] (3)				
	0.01	9 (1)	100 (1)				
Amphetamine sulphate	5.0	[illegible] ([illegible])	40 (3)				
	2.5	[illegible] (3)	[illegible] (4)				
"	1.0	5[illegible] ([illegible])	[illegible] ([illegible])	45 ([illegible])	[illegible] ([illegible])	[illegible] (4)	16 (4)
	0.5	[illegible] (1)	[illegible] ([illegible])			[illegible] (4)	[illegible] (4)
	0.25	[illegible] (5)	[illegible] ([illegible])	[illegible] (1)	[illegible] (1)	44 (4)	[illegible] (4)
	0.1		91 (1)	[illegible] (1)	[illegible] (1)		
Potassium cyanide	0.02/hr*	[illegible] ([illegible])	[illegible] ([illegible])			[illegible] (1)	100 (1)
"	0.01/hr	[illegible] (5)	[illegible] ([illegible])	100 (2)	100 ([illegible])	[illegible] (4)	[illegible] (4)
"	0.005/hr	[illegible] (4)	[illegible] (4)			54 ([illegible])	[illegible] ([illegible])
"	0.002/hr	71 (5)	81 ([illegible])	100 (1)	[illegible] (1)	[illegible] (4)	[illegible] (4)
"	0.001/hr	[illegible] (1)	[illegible] (5)				

* Potassium cyanide is added at one hour intervals


%
100
80
60
40
20
0
Inhibition of noradrenaline metabolism
A
1
KCN
I 0.01 0.05 0.1 0.2 0.5 mg/ml
A 0.25 0.5 1.0 2.0 2.5 5.0 mg/ml
KCN 1 2 5 10 20 × 10^3 mg/ml


%
100
80
60
40
20
0
Inhibition of adrenaline metabolism
A
KCN
1
I 0.01 0.05 0.1 0.2 0.5 mg/ml
A 0.25 0.5 1.0 2.0 2.5 5.0 mg/ml
KCN 1 2 5 10 20 × 10^3 mg/ml

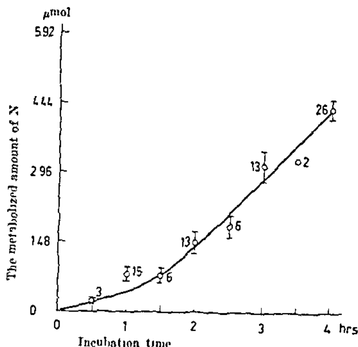


Figure 5
The metabolism of noradrenaline (N) in bovine heart muscle homogenate without the addition of enzyme inhibitors
The enzyme activity of 10 ml of 10 % (w/v) homogenate is expressed as µmol of N metabolized during incubation and oxidation at 37°C 5.92 µmol of N was added before incubation The figures show the number of single determinations on which the mean values are based The vertical lines express s e m

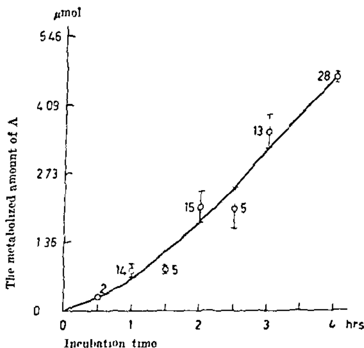
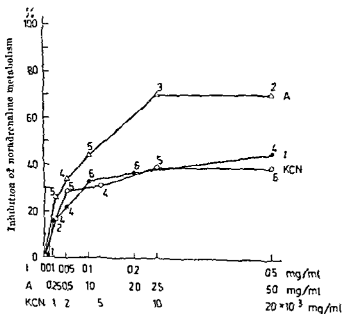


Figure 6
The metabolism of adrenaline (A) in bovine heart muscle homogenate without the addition of enzyme inhibitors
The enzyme activity of 10 ml of 10 % homogenate is expressed as µmol of A metabolized during incubation and oxidation at 37°C 5.46 µmol of A was added before incubation The figures show the number of single determinations on which the mean values are based The vertical lines express s e m


%
100
80
60
40
20
0
Inhibition of noradrenaline metabolism
A
I
KCN
I 001 005 01 02 05 mg/ml
A 025 05 10 20 25 50 mg/ml
KCN 1 2 5 10 20×10 3 mg/ml

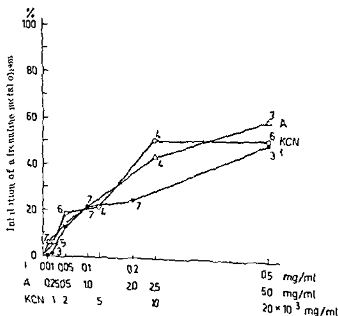

%
100
80
60
40
20
0
A
KCN
I 001 005 01 02 05 mg/ml
A 025 05 10 20 25 50 mg/ml
KCN 1 2 5 10 20×10 3 mg/ml

Figure 7

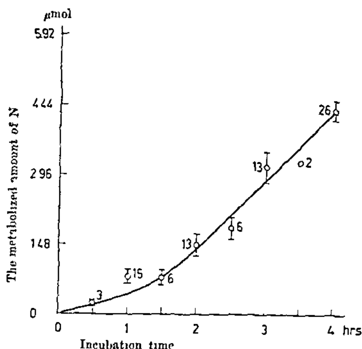


Figure 5
The metabolism of noradrenaline (N) in bovine heart muscle homogenate without the addition of enzyme inhibitors
The enzyme activity of 10 ml of 10 % (w/v) homogenate is expressed as µmol of N metabolized during incubation and oxidation at 37°C 5.92 µmol of N was added before incubation The figures show the number of single determinations on which the mean values are based The vertical lines express s e m

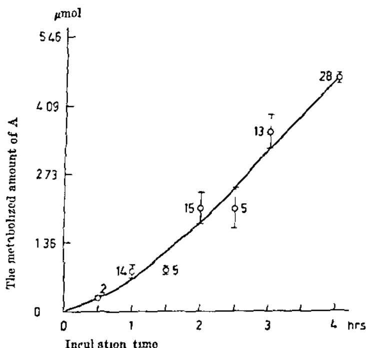
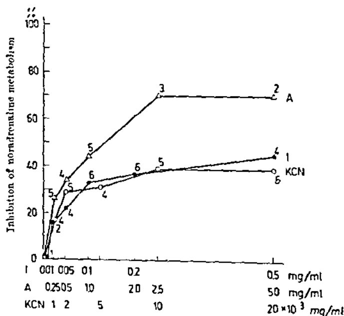


Figure 6
The metabolism of adrenaline (A) in bovine heart muscle homogenate without the addition of enzyme inhibitors
The enzyme activity of 10 ml of 10 % homogenate is expressed as µmol of A metabolized during incubation and oxidation at 37 C 5.46 µmol of A was added before incubation The figures show the number of single determinations on which the mean values are based The vertical lines express s e m


Inhibition of noradrenaline metabolism
%
100
80
60
40
20
0
A
1
KCN
I 001 005 01 02 05 mg/ml
A 0.25 0.5 1.0 2.0 2.5 5.0 mg/ml
KCN 1 2 5 10 20×10^{-3} mg/ml

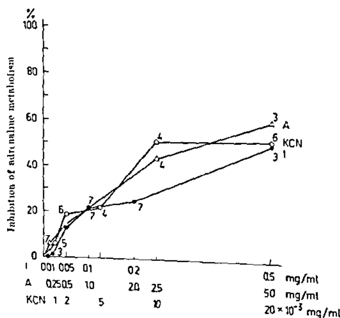

Inhibition of adrenaline metabolism
%
100
80
60
40
20
0
A
KCN
1
I 001 005 01 02 05 mg/ml
A 0.25 0.5 1.0 2.0 2.5 5.0 mg/ml
KCN 1 2 5 10 20×10^{-3} mg/ml

Figure 7

In the studies with *iproniazid* the highest inhibitor concentration was 0.5 mg/ml. When greater iproniazid concentrations were used there occurred an increased consumption of iodine in connection with the iodine oxidation reaction disturbing the colour reaction. Therefore the maximum effect of iproniazid on the enzymatic metabolism of noradrenaline and adrenaline cannot be measured in tests based on this colour reaction. Iproniazid (0.5 mg/ml) inhibited the *normal enzymatic activity metabolizing* noradrenaline and adrenaline in heart muscle tissue by 45 % and 50 % respectively. At the concentrations of 0.01 mg/ml and 0.02 mg/ml *it was no more capable of inhibiting the activity metabolizing noradrenaline* and adrenaline.

Amphetamine (5 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline *and adrenaline by 71 % and 60 % respectively.* This was the maximum inhibition. At a concentration of 0.25 mg/ml it still inhibited the activity of the suspension to some extent.

Potassium cyanide produced a clear inhibition of the enzymatic metabolism of noradrenaline and adrenaline in heart muscle tissue but not in any other tissues. Inhibition was statistically significant at potassium cyanide concentrations of 0.005—0.02 mg/ml ($P < 0.05$) when added at one hour intervals. A slight inhibition of the enzymatic metabolism of noradrenaline and adrenaline was already produced by adding 0.001 mg/ml of potassium cyanide to heart muscle suspensions at one hour intervals. An addition of 0.002 mg/ml potassium cyanide at one hour intervals inhibited noradrenaline and adrenaline metabolism by 39 % and 52 % respectively.

2. *Skeletal Muscle*

In a 10 % (w/v) homogenate prepared from bovine hind legs 0.2 μmol from 5.92 μmol noradrenaline (4 %) and 0.3 μmol from 5.46 μmol adrenaline (6 %) were metabolized during the 4 hour incubation. Skeletal muscle showed a very low enzymatic activity on noradrenaline and adrenaline. *Studies with specific enzyme inhibitors* were not considered necessary for this tissue.

3. *Uterine Tissue*

The endometrium was removed and the tissue homogenate was prepared as before. Ten single measurements were carried out with tissue suspensions made from seven different uteri. On the average 73 ± 2.3 % (4.3 μmol) of 5.92 μmol noradrenaline and 74 ± 2.9 % (4.0 μmol) of 5.46 μmol adrenaline were metabolized during the 4 hour incubation.

Enzyme Inhibitors

An incubation time of 4 hours was chosen for the studies with enzyme inhibitors. Figure 8 represents the results from seven different series of experiments.

Iproniazid (0.5 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in uterine tissue by 72 %. At a concentration of 0.05 mg/ml it inhibited the activity of uterine tissue by 10 %.

Amphetamine (1 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline by 75 % and 87 % respectively. An effective inhibition of the normal metabolism of both noradrenaline and adrenaline was still produced in uterine tissue by an amphetamine concentration which was only one fourth of the above concentration.

The *potassium cyanide* induced inhibition of the normal enzymatic metabolism of noradrenaline and adrenaline in uterine tissues was negligible. The effect did not increase linearly with the increase in the cyanide concentration. The maximum inhibition 12 % for noradrenaline and 11 % for adrenaline was reached when adding 0.01 mg/ml potassium cyanide at one hour intervals during the incubation. Such a small inhibition of the normal metabolism is not necessarily due to enzyme inhibition but could be produced by inhibition of autoxidation.

4. *Intestinal Wall*

Bovine small intestine was washed with water and its mucous membrane was removed by scraping, after which the tissue suspension was made in the usual way. The enzyme activity of the wall of the small intestine appeared to be quite low. On the average 56 ± 8.6 % (3.3 µmol) from the added 5.92 µmol noradrenaline and 51 ± 7.5 % (2.8 µmol) from the added 5.46 µmol adrenaline were metabolized during the 4 hour incubation. This was the mean of 4 series of experiments.

Enzyme Inhibitors

An incubation time of 4 hours was chosen for the studies with the enzyme inhibitors (fig. 9).

Iproniazid (0.2 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in intestinal tissue by 54 % and 56 % respectively. An inhibition of 22 % and of 21 % for noradrenaline and adrenaline respectively was still produced by an iproniazid concentration which was only one fourth of the above.

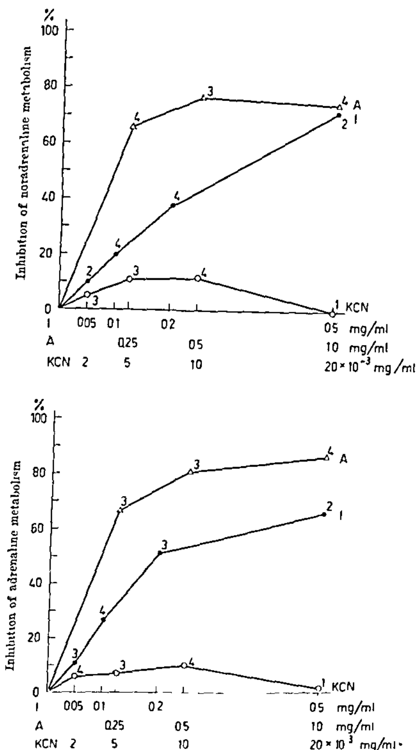
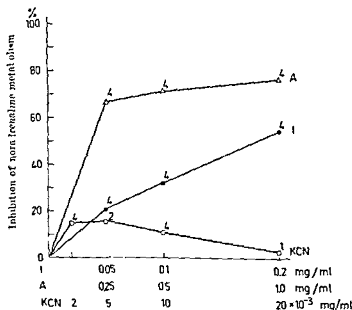


Figure 8

Inhibition of the enzymatic metabolism of noradrenaline and adrenaline when incubated at different inhibitor concentrations for 4 hours at 37°C in 10 ml of 10 % (w/v) bovine *uterus suspension*. Without inhibitors 4.3 μmol of added 5.02 μmol noradrenaline and 4.0 μmol of added 5.46 μmol adrenaline, on the average, had been metabolized. The inhibitor induced reduction has been calculated in per cent of the metabolized amount. The number of single determinations on which the mean values are based has been shown by figures. Abbreviations: A = amphetamine sulphate, I = iproniazid phosphate and KCN = potassium cyanide.


%
100
80
60
40
20
0
Inhibition of noradrenaline metabolism
A
I
KCN
I 0.05 0.1 0.2 mg/ml
A 0.25 0.5 1.0 mg/ml
KCN 2 5 10 20×10^{-3} mg/ml

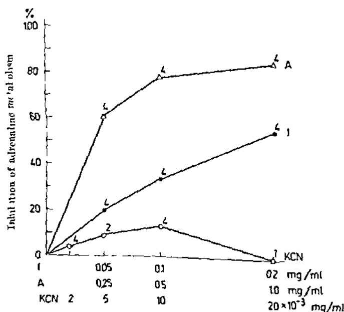

%
100
80
60
40
20
0
Inhibition of adrenaline metabolism
A
I
KCN
I 0.05 0.1 0.2 mg/ml
A 0.25 0.5 1.0 mg/ml
KCN 2 5 10 20×10^{-3} mg/ml

Figure 9

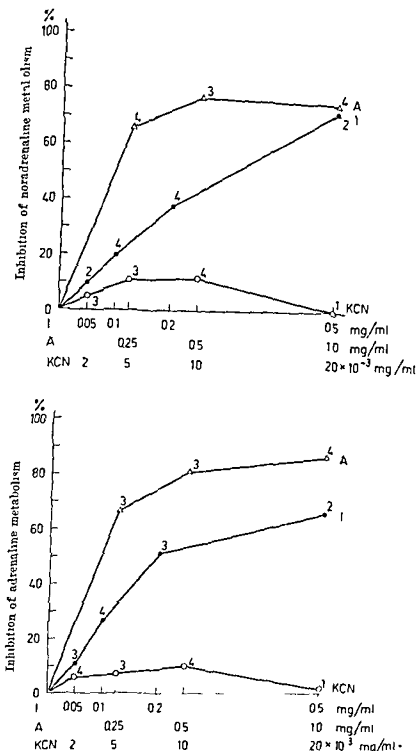


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6 Lung

The lung tissue suspensions were prepared avoiding the *bronchi* The metabolism of noradrenaline and adrenaline in the suspensions was quite rapid *On the average* 5 5 μmol (92 %) of 5 92 μmol noradrenaline and 4 8 μmol (88 %) of 5 46 μmol adrenaline were already metabolized during the 2 hour incubation (mean of two series of determinations)

Enzyme Inhibitors

An incubation time of two hours was chosen for the studies with enzyme inhibitors (fig 11)

Iproniazid (0 5 mg/ml) inhibited the normal enzyme activity meta bolizing noradrenaline and adrenaline in lung tissue *in vitro* by 47 % and 55 % respectively Practically no inhibition was produced by an iproniazid concentration which was one tenth of the above level (4 % for noradrenaline and 6 % for adrenaline)

Amphetamine inhibited the normal enzyme activity metabolizing nor adrenaline and adrenaline much to the same extent as iproniazid Still at the highest amphetamine concentration (1 mg/ml) the inhibition (55 % for noradrenaline and 62 % for adrenaline) was somewhat greater than that produced by iproniazid

Potassium cyanide could not inhibit the enzyme activity metabolizing noradrenaline and adrenaline in lung tissue

7 Brain

The suspensions were prepared using tissue from no particular part of the brain The enzyme activity in the suspensions was comparatively low On the average 68 ± 1 4 % (4 0 μmol) of 5 92 μmol noradrenaline and 62 ± 2 4 % (3 4 μmol) of 5 46 μmol adrenaline were metabolized during the 4 hour incubation (mean of three series of determinations)

Enzyme Inhibitors

An incubation time of 4 hours was chosen for the studies with enzyme inhibitors Figure 12 shows the means from the parallel measurements on three bovine brain tissues

Iproniazid (0 2 mg/ml) inhibited the normal enzyme activity meta bolizing noradrenaline and adrenaline in brain tissue by 29 % and 44 %

The *amphetamine* induced inhibition of the normal enzymatic metabolism of noradrenaline and adrenaline was even greater than that produced by iproniazid. At a concentration of 1 mg/ml it inhibited 74 % and 84 % of the metabolism of noradrenaline and adrenaline respectively.

Potassium cyanide produced only an insignificant inhibition of the normal enzyme activity of the intestinal wall on noradrenaline and adrenaline. The inhibition induced by potassium cyanide did not correspond to the normal progress of the enzyme inhibition which proceeds with the increase in the inhibitor concentration. The maximum inhibition was 16 % for noradrenaline and 13 % for adrenaline.

5. Spleen

After the removal of the capsule, bovine spleen tissue homogenate was made in the usual way. The series of experiments were performed using three different spleen tissues. On the average 53 ± 9.7 % (3.1 µmol) of 5.92 µmol noradrenaline and 60 ± 5.7 % (3.3 µmol) of 5.46 µmol adrenaline were metabolized during the 4 hour incubation.

Enzyme Inhibitors

An incubation time of 4 hours was chosen for the studies with the enzyme inhibitors (fig. 10).

Iproniazid (0.2 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in the spleen tissue by 73 % and 47 % respectively. An inhibition of 12 % and 17 % for noradrenaline and adrenaline respectively was produced by an iproniazid concentration which was one fourth of the above level.

Amphetamine (1 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in the spleen *in vitro* by 73 % and 47 % respectively. An inhibition of 45 % and 28 % for noradrenaline and adrenaline respectively was produced by an amphetamine concentration which was one fourth of the above level.

The *potassium cyanide* induced inhibition of the normal enzyme activity metabolizing catechol amines in the spleen was of a very small extent. The degree of inhibition did not correspond to the increase in the inhibitor concentration. The maximum inhibition, 6 % for noradrenaline and 11 % for adrenaline was reached when adding 0.01 mg/ml potassium cyanide at one hour intervals to the spleen homogenate.

6 *Lung*

The lung tissue suspensions were prepared avoiding the bronchi The metabolism of noradrenaline and adrenaline in the suspensions was quite rapid On the average 5 5 μmol (92 %) of 5 92 μmol noradrenaline and 4 8 μmol (88 %) of 5 46 μmol adrenaline were already metabolized during the 2 hour incubation (mean of two series of determinations)

Enzyme Inhibitors

An incubation time of two hours was chosen for the studies with enzyme inhibitors (fig 11)

Iproniazid (0 5 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in lung tissue *in vitro* by 47 % and 55 % respectively Practically no inhibition was produced by an iproniazid concentration which was one tenth of the above level (4 % for noradrenaline and 6 % for adrenaline)

Amphetamine inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline much to the same extent as iproniazid Still at the highest amphetamine concentration (1 mg/ml) the inhibition (55 % for noradrenaline and 62 % for adrenaline) was somewhat greater than that produced by iproniazid

Potassium cyanide could not inhibit the enzyme activity metabolizing noradrenaline and adrenaline in lung tissue

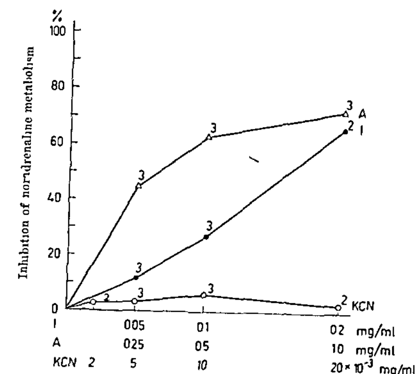
7 *Brain*

The suspensions were prepared using tissue from no particular part of the brain The enzyme activity in the suspensions was comparatively low On the average 68 ± 1 4 % (4 0 μmol) of 5 92 μmol noradrenaline and 62 ± 2 4 % (3 4 μmol) of 5 46 μmol adrenaline were metabolized during the 4 hour incubation (mean of three series of determinations)

Enzyme Inhibitors

An incubation time of 4 hours was chosen for the studies with enzyme inhibitors Figure 12 shows the means from the parallel measurements on three bovine brain tissues

Iproniazid (0 2 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in brain tissue by 29 % and 44 %



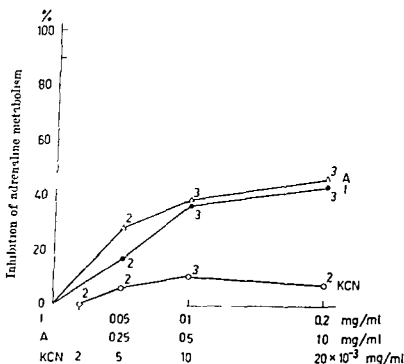
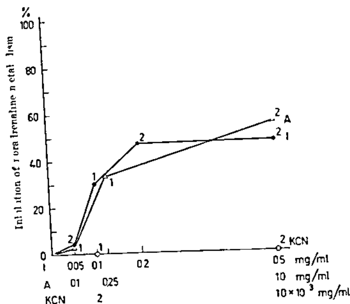


Figure 10

Inhibition of the enzymatic metabolism of noradrenaline and adrenaline when incubated at different inhibitor concentrations for 4 hours at 37° C in 10 ml of 10 % (w/v) bovine *spleen* suspension. Without inhibitors, 3.1 μmol of added 5.92 μmol noradrenaline and 3.3 μmol of added 5.46 μmol adrenaline, on the average, had been metabolized. The inhibitor induced reduction has been calculated in per cent of the metabolized amount. *The number of single determinations on which the mean values are based has been shown by figures.* Abbreviations: A = amphetamine sulphate, I = iproniazid phosphate and KCN = potassium cyanide.



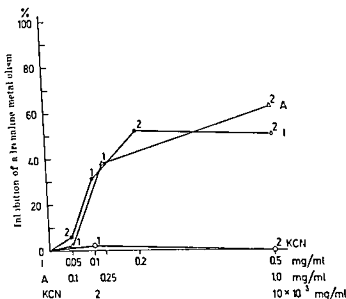


Figure 11

Inhibition of the enzymatic metabolism of noradrenaline and adrenaline when incubated at different inhibitor concentrations for 2 hours at 3 °C in 10 ml of 10 % (w/v) bovine *lung* suspension Without inhibitors 5.5 μmol of added 5.9 μmol noradrenaline and

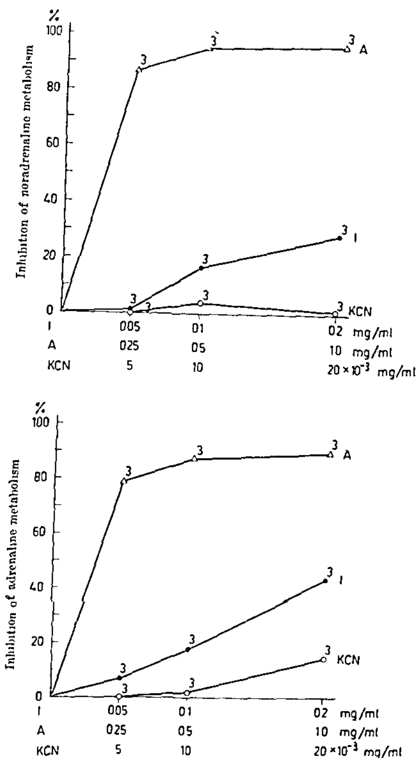
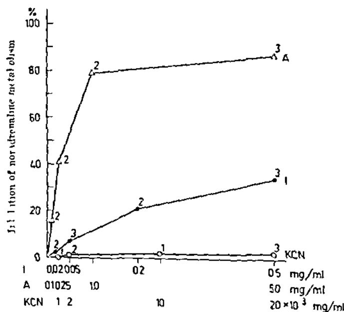


Figure 12

Inhibition of the enzymatic metabolism of noradrenaline and adrenaline when incubated at different inhibitor concentrations for 4 hours at 37° C in 10 ml of 10 % (w/v) bovine brain suspension. Without inhibitors, 4.0 µmol of added 5.92 µmol noradrenaline and 3.4 µmol of added 5.46 µmol adrenaline, on the average, had been metabolized. The inhibitor induced reduction has been calculated in per cent of the metabolized amount. The number of single determinations on which the mean values are based has been shown by figures. Abbreviations: A = amphetamine sulphate, I = iproniazid phosphate and KCN = potassium cyanide.


%
100
80
60
40
20
0
I 0.02 0.05 0.2 0.5 mg/ml
A 0.1 0.25 1.0 5.0 mg/ml
KCN 1 2 10 20 × 10 3 mg/ml
A
I
KCN

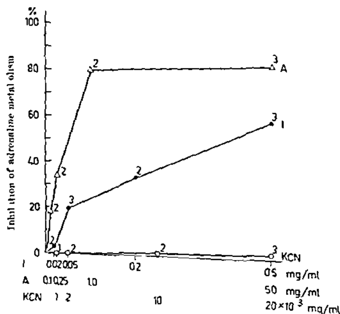

%
100
80
60
40
20
0
Inhibition of adrenaline metabolism
I 0.02 0.05 0.2 0.5 mg/ml
A 0.1 0.25 1.0 5.0 mg/ml
KCN 1 2 10 20 × 10 3 mg/ml
A
I
KCN

Figure 13

respectively One fourth of the above iproniazid concentration produced no clear inhibition

Amphetamine induced an exceptionally great inhibition of the normal enzymatic metabolism of noradrenaline and adrenaline in the brain tissue suspension Thus 1 mg/ml inhibited the noradrenaline and adrenaline metabolism by 97 % and 90 % respectively Extensive inhibition was observed in all the three series

Potassium cyanide produced no clear inhibition of the normal enzyme activity metabolizing noradrenaline and adrenaline in the brain tissue However, an inhibition of 15 % was observed in adrenaline metabolism when 0 01 mg/ml/hr potassium cyanide was added during the incubation Since no similar inhibition was seen in connection with noradrenaline metabolism, the inhibition of adrenaline metabolism may be due to exceptionally extensive autoxidation Still only 9 % of adrenaline, on an average, was destroyed in preheated brain tissue homogenates during the 4 hour incubation

8 Kidney

The metabolism of noradrenaline and adrenaline was quick in bovine kidney tissue homogenates On the average, 4 6 ± 0 10 μmol (77 %) of 5 92 μmol noradrenaline and 3 8 ± 0 10 μmol (70 %) of 5 46 μmol adrenaline were metabolized during the 1 hour incubation

Enzyme Inhibitors

An incubation time of 1 hour was chosen for the studies with enzyme inhibitors (fig 13)

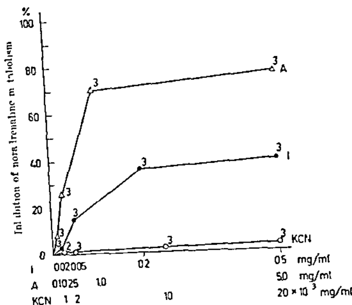
Iproniazid (0 5 mg/ml) inhibited noradrenaline metabolism by 34 % and that of adrenaline by 59 % 0 02 mg/ml produced no inhibition of noradrenaline and adrenaline metabolism

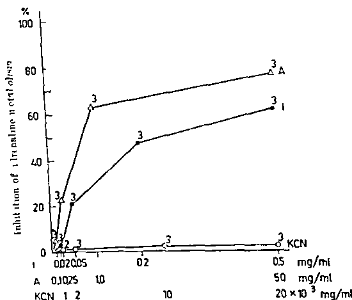
Amphetamine (5 mg/ml) inhibited the normal enzyme activity metabolizing noradrenaline and adrenaline in kidney homogenate by 86 % and 83 % respectively The corresponding inhibition was 16 % and 18 % respectively at an amphetamine concentration of 0 1 mg/ml

Potassium cyanide had no inhibitory effect on noradrenaline and adrenaline metabolism in kidney tissue

9 Liver

On the average, 4 3 ± 0 15 μmol (72 %) of 5 92 μmol noradrenaline and 4 2 ± 0 20 μmol (77 %) adrenaline were metabolized in bovine liver tissue homogenates during the 1 hour incubation


%
100
80
60
40
20
0
Inhibition of noradrenaline metabolism
3 A
3 I
3 KCN
I 0.02 0.05 0.2 0.5 mg/ml
A 0.1 0.25 1.0 5.0 mg/ml
KCN 1 2 10 20 × 10 3 mg/ml


%
100
80
60
40
20
0
Inhibition of adrenaline metabolism
3 A
3 I
3 KCN
I 0.02 0.05 0.2 0.5 mg/ml
A 0.1 0.25 1.0 5.0 mg/ml
KCN 1 2 10 20 × 10 3 mg/ml

According to DIXON (1953) the MICHAELIS constant (K_m) in the enzyme reaction was calculated to be 1.7×10^{-3} M when incubating noradrenaline for ½ hour in 4 different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml).

Enzyme Inhibitors

An incubation time of 1 hour was chosen for the studies with enzyme inhibitors. The results are to be seen in fig. 14.

Iproniazid (0.5 mg/ml) inhibited both noradrenaline and adrenaline metabolism by 61 %. An iproniazid concentration of 0.02 mg/ml only produced an insignificant inhibition of noradrenaline and adrenaline metabolism.

Amphetamine (5 mg/ml) inhibited the normal enzymatic metabolism of both noradrenaline and adrenaline by 76 %. An amphetamine concentration of 0.1 mg/ml produced no clear inhibition of the metabolism.

Potassium cyanide had no inhibitory effects on noradrenaline or adrenaline metabolism in liver tissue homogenates.

2. Biological Determination

1. *Method for Incubation and Biological Determination*

In chemical determinations with the iodine oxidation reaction the concentrations of noradrenaline and adrenaline had to be fairly high (0.1 mg/ml). To throw light upon the enzymatic activity in small substrate concentrations studies were performed using biological tissue concentrations or adding only small amounts of noradrenaline (0.5—3.0 μM) as a substrate. These studies were made using only noradrenaline as a substrate. The metabolic rate and its inhibition were greatly similar for noradrenaline and adrenaline respectively in different tissues at higher concentrations. Noradrenaline is an important mediator of the sympathetic nerve impulses. In most tissues except in adrenals adrenaline comprises only a few per cents of the total amount of the two amines (e.g. HOLTZ *et al.* 1951, HOKFELT 1951, VON EULER 1956, PEKKARINEN *et al.* 1962). Consequently comparable results could be expected from studying only the tissue noradrenaline contents. By doing so it was also possible to manage with fewer biological determinations.

At low noradrenaline concentrations the method was similar to that used at higher substrate concentrations. Bovine tissue homogenates were incubated in a continuous oxygen stream at 37°C to produce enzymatic metabolism. The noradrenaline content determined after the incubation

was compared with that obtained before the incubation The inhibitors were added to the tissue homogenate before the incubation In case the substrate had to be added so as to render the determinations possible the tissue suspension was preincubated with enzyme inhibitors for half an hour before adding the noradrenaline In such cases the endogenous noradrenaline of the tissues could be partly metabolized during the preincubation Thus the noradrenaline contents of the samples with the substrate added before the preincubation are not quite correct as control values Since however the contents of endogenous noradrenaline were quite small even in these tissues the preincubation does not produce any significant mistake

Tissue samples were minced for 1—1.5 min in a homogenisator To 30 g of tissue was added 80 ml of distilled water The pH of the tissue homogenate was adjusted by means of a pH Radiometer to 7 4 with 0 25 N NaOH The volume of the tissue homogenate was made up to 150 ml with distilled water The homogenate was then divided into 6 test tubes with 25 ml for each Enzyme inhibitors were added The volume of the homogenate was made up to 30 ml with distilled water The final tissue suspension was thus 17 % (w/v) The incubation at 37°C was performed as in connection with the greater substrate concentrations Potassium cyanide was added at one hour intervals to counteract its possible destruction during incubation Endogenous amounts of noradrenaline were considered sufficient for the determinations in heart and spleen tissue suspensions but small amounts of noradrenaline were added to the other tissues to facilitate the biological noradrenaline determinations These additions were made after the tissue samples had been preincubated with enzyme inhibitors at 37°C with simultaneous oxygenation The preincubation lasted for some 30 min

After the incubation the tissue proteins were precipitated by adding 8 ml of 15 % trichloroacetic acid (HOKFELT 1951) and shaking the test tubes carefully After this the suspension was stored in a refrigerator till the following day (in most cases) Following the shaking they were centrifuged or filtered and the supernatant liquid was carefully retained 2 % aluminium sulphate equalling 1/5 of the whole volume of the sample (7 6 ml) was then added to the filtrate (SHAW 1938 v EULER 1956) The pH of the filtrate was raised up to 8 5 (PEKKARINEN 1948) by adding 2 N NaOH with a resulting precipitation of aluminium hydroxide The alkaline suspension was shaken vigorously for some 2 min after which the samples were centrifuged and the aluminium hydroxide precipitate was retained The precipitate was washed with slightly alkaline distilled water (pH 8 0—8 5 produced by NaOH) The precipitate was immediately dissolved into a minimum volume of 2 N H_2SO_4 (e g into 0 5 ml) Checking with 0 1 %

methyl orange solution and indicator paper the pH of the solution was raised up to 3 with 0.25 N NaOH. A 4-fold volume of alcohol-acetone mixture (ana partes) was added to the solution (VON EULER 1956). After shaking the test tubes were stored in a refrigerator for 12—24 hours during which time the salts were precipitated. The alcohol and acetone were evaporated under reduced pressure in 35°—37°C waterbath or in a vacuum oven at 37°C. Before the determinations the pH of the samples was made up to 3.5 with 0.25 N NaOH. The final volume (1.5—5 ml) was obtained by adding distilled water.

The noradrenaline determinations were performed using the well known method based on the cat's blood pressure response (VON EULER 1956). The cats were anesthetized with chloralose (60 mg/kg), tracheal cannulization was performed and mechanical respiration was commenced using a *Starling Ideal pump*. *The blood pressure* was registered with mercury manometer connected to the carotid artery by means of a glass cannula. The injections were *given through a cannula fastened to the femoral vein.* In order to stabilize the blood pressure and to sensitize the response the cat was given 2 mg/kg atropine sulphate s.c., 6—8 mg/kg cocaine hydrochloride i.m. and 0.1 mg/kg ergotamine tartrate also i.m. To potentiate the blood pressure reaction 3 mg/kg mepyramine maleate (Anthisan®) was given i.m. (KARKI 1956). In case the blood pressure was considerably elevated at the beginning of the experiment and noradrenaline injections were *not capable of producing the expected rise in it, the blood pressure* was decreased by means of small amounts (2—4 mg/kg) of intramuscularly given hexamethonium (Vegolysen®) (KARKI 1956). The adrenaline induced blood pressure response was also tested in every cat. In case the reaction proved negative, repeated adrenaline injections were given and somewhat larger amounts of ergotamine were administered to render it positive. The blood pressure level for a cat was usually observed to be 100—130 mmHg during the experiments. 0.25—0.5 ml of the tissue sample was *injected into* the animal through the vein cannula. The cannula was washed with 1.5 ml *of warm newly made 0.9 % sodium chloride 5 % glucose* solution (suggested by PEKKARINEN). Every sample was tested with 1—4 injections of l-noradrenaline bitartrate of known concentration (calculated as noradrenaline base). As much noradrenaline was injected as was needed to produce a rise in the blood pressure equalling that induced by the sample. The sample could also be estimated with two doses of standard noradrenaline which gave a blood pressure response close to that of the sample. To increase the accuracy when *measuring small amounts of noradrenaline* almost all samples were injected twice. The ratio between the blood pressure reactions *produced* by adrenaline and noradrenaline was usually 0.1—0.25. In a number of cases the injection of small amounts of adrenaline (0.2 µg)

brought about no clear increase in the blood pressure. Since tissue adrenaline contents are usually only some per cents of the whole amount of catechols in them, e.g. bovine or rat heart muscle 7—23 % adrenaline (e.g. HOLTZ *et al.* 1951, PEKKARINEN *et al.* 1952) practically the whole blood pressure reaction measured was produced by noradrenaline.

The tissue suspension was 17 % (w/v) when studying biological amounts of noradrenaline, whereas the corresponding value was 10 % (w/v) when determining larger noradrenaline and adrenaline amounts in the previous tests. In the first place this was due to the fact that at least 5 g of tissue was needed to yield the minimum measurable amount of noradrenaline. On the other hand, technical reasons prevented the use of a volume of 50 ml for incubation. The greater metabolism rate of noradrenaline in biological studies is thus also partly explained by the greater amount of the enzyme source.

To find out the methodical error the noradrenaline contents of heart muscle tissue were determined using two different tissue samples and performing four simultaneous determinations. The results of the two experiments are as follows:

Tissue 1	Noradrenaline µg/g ± s.e.m.	*Tissue 2*	Noradrenaline µg/g ± s.e.m.
	0.16		0.49
	0.1		0.54
	0.18		0.45
	0.24		0.51
	0.19 ± 0.018		0.50 ± 0.014

The thermolability of endogenous noradrenaline excluded the heating of the samples done in the other studies to destroy the enzymatic activity of the samples. The inactivation rate of noradrenaline may be slightly affected by autoxidation in the studies made with biological or near biological noradrenaline concentrations. The probable part played by autoxidation could not be ascertained.

I. Results (Table 8)

1. *Heart Muscle*

Bovine heart muscle tissue contained 0.16—0.92 µg noradrenaline/g in the seven series of determinations made. The average was 0.45 µg/g. The substrate concentration averaged thus 0.47 µM in the incubation sus

methyl orange solution and indicator paper the pH of the solution was raised up to 3 with 0.25 N NaOH. A 4-fold volume of alcohol-acetone mixture (ana partes) was added to the solution (VON EULER 1956). After shaking, the test tubes were stored in a refrigerator for 12—24 hours, during which time the salts were precipitated. The alcohol and acetone were evaporated under reduced pressure in 35°—37°C waterbath or in a vacuum oven at 37°C. Before the determinations the pH of the samples was made up to 3.5 with 0.25 N NaOH. The final volume (1.5—5 ml) was obtained by adding distilled water.

The noradrenaline determinations were performed using the well-known method based on the cat's blood pressure response (VON EULER 1956). The cats were anesthetized with chloralose (60 mg/kg), tracheal cannulization was performed and mechanical respiration was commenced using a Starling Ideal pump. The blood pressure was registered with mercury manometer connected to the carotid artery by means of a glass cannula. The injections were given through a cannula fastened to the femoral vein. In order to stabilize the blood pressure and to sensitize the response, the cat was given 2 mg/kg atropine sulphate s.c., 6—8 mg/kg cocaine hydrochloride i.m., and 0.1 mg/kg ergotamine tartrate also i.m. To potentiate the blood pressure reaction 3 mg/kg mepyramine maleate (Anthisan®) was given i.m. (KARKI 1956). In case the blood pressure was considerably elevated at the beginning of the experiment and noradrenaline injections were not capable of producing the expected raise in it, the blood pressure was decreased by means of small amounts (2—4 mg/kg) of intramuscularly given hexamethonium (Vegolysen®) (KARKI 1956). The adrenaline induced blood pressure response was also tested in every cat. In case the reaction proved negative, repeated adrenaline injections were given and somewhat larger amounts of ergotamine were administered to render it positive. The blood pressure level for a cat was usually observed to be 100—130 mmHg during the experiments. 0.25—0.5 ml of the tissue sample was injected into the animal through the vein cannula. The cannula was washed with 1.5 ml of warm, newly made 0.9 % sodium chloride 5 % glucose solution (suggested by PEKKARINEN). Every sample was tested with 1—4 injections of l-noradrenaline bitartrate of known concentration (calculated as noradrenaline base). As much noradrenaline was injected as was needed to produce a raise in the blood pressure equalling that induced by the sample. The sample could also be estimated with two doses of standard noradrenaline which gave a blood pressure response close to that of the sample. To increase the accuracy when measuring small amounts of noradrenaline almost all samples were injected twice. The ratio between the blood pressure reactions produced by adrenaline and noradrenaline was usually 0.1—0.25. In a number of cases the injection of small amounts of adrenaline (0.2 μg)

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The tissue suspension was 17 % (w/v) when studying biological amounts of noradrenaline, whereas the corresponding value was 10 % (w/v) when determining larger noradrenaline and adrenaline amounts in the previous tests. In the first place this was due to the fact that at least 5 g of tissue was needed to yield the minimum measurable amount of noradrenaline. On the other hand, technical reasons prevented the use of a volume of 50 ml for incubation. The greater metabolism rate of noradrenaline in biological studies is thus also partly explained by the greater amount of the enzyme source.

To find out the methodical error the noradrenaline contents of heart muscle tissue were determined using two different tissue samples and performing four simultaneous determinations. The results of the two experiments are as follows:

Tissue 1	Noradrenaline µg/g ± s.e.m.	*Tissue 2*	Noradrenaline µg/g ± s.e.m.
	0.16		0.45
	0.17		0.54
	0.18		0.48
	0.24		0.51
	0.19 ± 0.018		0.50 ± 0.014

The thermolability of endogenous noradrenaline excluded the heating of the samples done in the other studies to destroy the enzymatic activity of the samples. The inactivation rate of noradrenaline may be slightly affected by autoxidation in the studies made with biological or near biological noradrenaline concentrations. The probable part played by autoxidation could not be ascertained.

P. *Results* (Table 6)

1. *Heart Muscle*

Bovine heart muscle tissue contained 0.16—0.92 µg noradrenaline/g in the seven series of determinations made. The average was 0.45 µg/g. The substrate concentration averaged thus 0.47 µM in the incubation sus-

Table 8

The metabolism of noradrenaline in bovine tissues at small, biological or near biological concentrations. The enzyme activity at different inhibitor concentrations

The tissue homogenate was 17 % (w/v). The incubation was carried out at 37°C with continuous oxygenation. T h e e n z y m a t i c a c t i v i t y i s e x p r e s s e d a s p e r c e n t o f t h e m a x i m u m m e t a b o l i s m (100 %) under incubation. In brackets the number of single determinations (n) on which the mean values are based. The substrate in heart and spleen homogenates is endogenous noradrenaline, in the other tissue homogenates an addition of noradrenaline has been done.

Tissue		Heart	Lung	Intestine	Uterus	Spleen	Liver	Kidney	Brain
The mean concentration of noradrenaline (μM)		0.47	1.0	1.0—3.0	2.0	0.37	0.4—1.0	1.0	0.5—1.0
Incubation time	Enzyme inhibitor mg/ml	% n	% n	% n	% n	% n	% n	% n	% n
3 hrs	Iproniazid phosphate 13	57 (2)	40 (2)		68 (2)				6 (4)
	7	70 (3)	71 (3)		65 (5)				3 (4)
	0.7	100 (3)	100 (3)		78 (4)				78 (4)
	Amphetamine sulphate 7	96 (2)	96 (2)		36 (5)				45 (2)
	0.7		99 (2)						46 (2)
	Potassium cyanide 0.07/hr *	94 (2)	100 (4)		96 (1)				100 (4)
	0.013/hr	97 (4)	100 (4)		98 (6)				100 (4)
2 hrs	Iproniazid phosphate 13	61 (2)	54 (2)	3 (2)		55 (3)	42 (2)	[illegible] (2)	26 (5)
	7	47 (5)	40 (3)	61 (2)		87 (4)	29 (8)	44 (4)	67 (4)
	0.7	90 (4)	98 (4)	100 (2)		90 ( )	86 (2)	92 (4)	77 (5)
	Amphetamine sulphate 7	60 (4)	92 (1)	100 (1)		85 (5)	60 (5)	81 (5)	47 (2)
	0.7	79 (1)	99 (1)	100 (1)		100 (3)	98 (1)	96 (1)	62 (2)
	Potassium cyanide 0.07/hr *	80 (2)	100 (1)	100 (2)		98 (4)	100 (5)	99 (4)	99 (4)
	0.013/hr	84 (7)	100 (4)	100 (2)		94 (4)	100 (2)	97 (6)	99 ([illegible])
1 hr	Iproniazid phosphate 13			9 (5)		3 (2)	16 (2)	48 (4)	
	7			44 (5)		0 (1)	72 (5)	9 (5)	
	0.7			76 (4)		98 (2)	90 (2)	71 (4)	
	Amphetamine sulphate 7			61 (4)		97 ( )	8 (4)	81 (2)	
	0.7			67 (4)		100 (1)	100 (1)	97 (4)	
	Potassium cyanide 0.07/hr *			100 (2)			100 (1)	100 (2)	
	0.01 /hr			97 ([illegible])		89 (3)	100 (4)	100 (6)	

In addition to the incubation times shown in the table, noradrenaline was also incubated in uterine tissue for 4 hours when the enzymatic activity was 61 % (4), 43 % (4) and 48 % (4) for the different iproniazid concentrations 69 % (4) for amphetamine (7 mg/ml) and 100 % (4) for the two potassium cyanide concentrations. The results of the ½ hour incubation in the small intestine were iproniazid 12 % (2), 10 % (1) and 98 % (2), amphetamine (0.7 mg/ml) 100 % (1) potassium cyanide 100 % (1) and 93 % (2).

* Potassium cyanide is added at one hour intervals

pension The metabolic rate varied in the different series When adding 15 μg noradrenaline in one heart tissue suspension the recovery percentages were 94 % and 98 % in two simultaneous determinations Two tissues out of four showed no active noradrenaline after an incubation of 2 hours Studies were carried out with enzyme inhibitors incubating the samples for 3 and for 2 hours The results are to be seen in table 8 The inhibition of the enzymatic activity in tissues by different inhibitors has been calculated in per cent The amount of noradrenaline which remained unmetabolized has not been included in the results

Enzyme Inhibitors

Iproniazid at a concentration of 7—13 mg/ml decreased the enzymatic metabolism of noradrenaline by heart muscle by 30—43 % ($P > 0.05$) A concentration of 0 7 mg/ml inhibited no more the enzyme activity of heart muscle tissue during the 3 hour incubation Incubation of noradrenaline for 2 hours at an iproniazid concentration of 7 mg/ml produced a clear inhibition (53 %) ($P < 0.01$) 90 % of noradrenaline was inactivated at an iproniazid concentration of 0 7 mg/ml ($P > 0.05$)

At a considerably high *amphetamine* concentration (7 mg/ml) the heart tissue metabolized almost all noradrenaline in 3 hours On the average the enzyme activity was reduced by 40 % during the 2 hour incubation The results varied considerably in this experiment ($P > 0.05$)

Potassium cyanide proved to be a poor inhibitor of noradrenaline metabolism under experimental conditions The greatest inhibition was produced during the 2 hour incubation at a cyanide concentration of 0 013 mg/ml when 84 % of the noradrenaline was inactivated The inhibition was significant ($P < 0.01$) However noradrenaline autoxidation may be involved

2 *Uterine Tissue*

Bovine uterine tissue (5 g) did not contain any clearly measurable amount of noradrenaline Accordingly noradrenaline (2 μg/g of tissue) was added to the homogenate before the incubation

During the incubation the noradrenaline concentration was thus 2 0 μM The control noradrenaline measurement gave 1 40 μg/g (70 %) recovery After the 3 hour incubation there remained only very small amounts (0 02 μg/g on the average) of noradrenaline in the uterine tissue samples After the 2 hour incubation almost all noradrenaline was still unmetabolized in one of the two uterine tissue samples

Table 8

The metabolism of noradrenaline in bovine tissues at small, biological or near biological, concentrations. The enzyme activity at different inhibitor concentrations

The tissue homogenate was 17 % (w/v). The incubation was carried out at 37°C with continuous oxygenation. The enzymatic activity is expressed as per cent of the maximum metabolism (100 %) under incubation. In brackets the number of single determinations (n) on which the mean values are based. The substrate in heart and spleen homogenates is endogenous noradrenaline, in the other tissue homogenates an addition of noradrenaline has been done.

Tissue		Heart	Lung	Intestine	Uterus	Spleen	Liver	Kidney	Brain
The mean concentration of noradrenaline (μM)		0.47	1.0	1.0—3.0	2.0	0.37	0.4—1.0	1.0	0.5—1.0
Incubation time	Enzyme inhibitor mg/ml	% n	% n	% n	% n	% n	% n	% n	% n
3 hrs	Iproniazid phosphate 13	57 (2)	40 (2)		38 (2)				6 (4)
	7	*70 (3)*	*71 (3)*		65 (5)				*3 (4)*
	0.7	100 (3)	100 (3)		75 (4)				78 (4)
	Amphetamine sulphate 7	96 (2)	96 (2)		56 (5)				65 (2)
	0.7		99 (2)						46 (2)
	Potassium cyanide 0.07/hr *	94 (2)	100 (4)		*96 (1)*				*100 (4)*
	0.013/hr	97 (4)	100 (4)		98 (6)				100 (4)
2 hrs	Iproniazid phosphate 13	61 (2)	54 (2)	3 (2)		55 (3)	42 (2)	23 (2)	26 (3)
	7	47 (5)	40 (3)	61 (2)		87 (4)	29 (8)	14 (1)	67 (4)
	0.7	90 (4)	98 (4)	100 (2)		90 ([illegible])	56 (2)	92 (4)	77 (5)
	Amphetamine sulphate 7	60 (4)	*92 (1)*	100 (1)		*85 (5)*	*66 (5)*	*81 (5)*	47 (2)
	0.7	79 (1)	99 (1)	100 (1)		100 (3)	98 (1)	96 (1)	62 (2)
	Potassium cyanide 0.07/hr *	80 (2)	100 (1)	100 (2)		98 (4)	100 (5)	99 (4)	99 (4)
	0.013/hr	84 (7)	100 (4)	100 (2)		94 (4)	100 (2)	97 (6)	[illegible]
1 hr	Iproniazid phosphate 13			9 (5)		3 (2)	16 (2)	48 (4)	
	7			44 (5)		60 (1)	32 (5)	39 (5)	
	0.7			*76 (4)*		98 (2)	90 (2)	71 (4)	
	Amphetamine sulphate 7			61 (4)		97 (3)	83 (4)	81 (2)	
	0.7			67 (4)		100 (1)	100 (1)	97 (4)	
	Potassium cyanide 0.07/hr *			100 (2)			100 (1)	100 (2)	
	0.013/hr			97 (3)		89 (3)	100 (4)	100 (6)	

In addition to the incubation times shown in the table, noradrenaline was *also incubated in uterine* tissue for 4 hours, when the enzymatic activity was 61 % (4), 13 % (4) and 18 % (4) for the different iproniazid concentrations, 69 % (4) for amphetamine (7 mg/ml) and 100 % (4) for the two potassium cyanide concentrations. The results of the ½ hour incubation in the small intestine were, iproniazid 12 % (2), 10 % (1) and 98 % (2), amphetamine (0.7 mg/ml) 100 % (1), potassium cyanide 100 % (1) and 93 % (2).

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* Potassium cyanide is added at one hour intervals

Potassium cyanide did not produce any significant inhibition of the enzymatic metabolism of noradrenaline in bovine intestinal tissue

4 *Spleen*

The noradrenaline contents of bovine spleen were 0 24 µg/g, 0 39 µg/g and 0 50 µg/g as measured using samples from three different test animals. A 3 µg/g addition of noradrenaline to a spleen homogenate gave a recovery percentage of 62 ± 5 % in a tissue where the average endogenous noradrenaline content was 0 39 µg/g No noradrenaline was added to the suspensions before the incubation and thus the metabolism of the endogenous noradrenaline in the spleen tissue was studied In 2 out of the 3 tissues studied all observable noradrenaline had been destroyed within the 2 hour incubation In one tissue homogenate only 6 % of the whole amount of the endogenous noradrenaline still remained after that time During the 1 hour incubation 100 % 89 % and 81 % of noradrenaline were inactivated in spleen tissues

Enzyme Inhibitors

The studies with enzyme inhibitors were performed using incubation times of 1 hour and 2 hours

Iproniazid (13 mg/ml) reduced the enzyme activity metabolizing noradrenaline in the spleen down to 55 % ($P < 0 05$) within 2 hours and down to 3 % within 1 hour Even a concentration of 0 7 mg/ml inhibited noradrenaline inactivation in a very small extent

Amphetamine (7 mg/ml) produced a 15 % decrease in the enzymatic inactivation of noradrenaline in bovine spleen tissue ($P < 0 01$) A ten fold smaller concentration of amphetamine had no inhibitory effects on the metabolism of noradrenaline

When *potassium cyanide* was used the inactivation level was 89—98 % The inhibition was not significant

5 *Lung*

Bovine lung tissue (5 g) did not contain any clearly measurable amount of noradrenaline Accordingly noradrenaline (1 µg/g of tissue) was added to the homogenate before the incubation The control measurement gave 0 59—0 72 µg/g (recovery 59—72 %) as the noradrenaline amount The substrate concentration was thus 1 0 µM during the incubation After the 2 hour incubation noradrenaline was completely destroyed in two

Enzyme Inhibitors

The studies with enzyme inhibitors were made using 3 and 4 hours incubation times.

Iproniazid (7—13 mg/ml) reduced the noradrenaline metabolizing activity of uterine tissue suspensions by some 50 % during the 3 hour incubation ($P < 0.02$). At a concentration of 0.7 mg/ml the enzyme activity was still 78 % ($P > 0.05$). A significant inhibition was also brought about by the highest iproniazid concentration during the 4 hour incubation.

Amphetamine (7 mg/ml) reduced the noradrenaline metabolism in the uterine tissue down to 56 % ($P < 0.02$) and to 69 % ($P > 0.05$) during the 3 and 4 hour incubation times respectively.

Potassium cyanide produced no reduction of the noradrenaline metabolism in the uterine tissue samples.

3. *Intestinal Wall*

Bovine small intestine contained some 0.01—0.03 μg noradrenaline/g, *an amount which is not easily measurable. Noradrenaline was added* 1—3 μg/g of tissue giving thus an incubation suspension of 1—3 μM. In two simultaneous control determinations the recovery percentages of noradrenaline were 73 % and 69 %. In two out of three experiments all noradrenaline had disappeared from intestine homogenate after the 1 hour incubation. Even after an incubation of half an hour 59 ± 2.1 % of the noradrenaline had been destroyed.

Enzyme Inhibitors

Studies with enzyme inhibitors were performed with incubation times of ½, 1 and 2 hrs.

At the highest *iproniazid* concentrations the noradrenaline inactivation was *greatly reduced even after the 2 hour incubation.* A concentration of 0.7 mg/ml did not clearly ($P > 0.05$) inhibit the noradrenaline inactivation in bovine small intestine tissue even during the shortest incubation time.

At a 7 mg/ml *amphetamine concentration 61 % of noradrenaline* was metabolized enzymatically within one hour, but in this case as well as in the other amphetamine concentrations the results varied so much ($P > 0.05$) that the inhibition of noradrenaline metabolism by amphetamine cannot be *regarded as shown conclusively* within the periods used in this study.

Potassium cyanide did not produce any significant inhibition of the enzymatic metabolism of noradrenaline in bovine intestinal tissue

4 Spleen

The noradrenaline contents of bovine spleen were 0 24 µg/g 0 39 µg/g and 0 50 µg/g as measured using samples from three different test animals A 3 µg/g addition of noradrenaline to a spleen homogenate gave a recovery percentage of 62 ± 5 % in a tissue where the average endogenous noradrenaline content was 0 39 µg/g No noradrenaline was added to the suspensions before the incubation and thus the metabolism of the endogenous noradrenaline in the spleen tissue was studied In 2 out of the 3 tissues studied all observable noradrenaline had been destroyed within the 2 hour incubation In one tissue homogenate only 6 % of the whole amount of the endogenous noradrenaline still remained after that time During the 1 hour incubation 100 % 89 % and 81 % of noradrenaline were inactivated in spleen tissues

Enzyme Inhibitors

The studies with enzyme inhibitors were performed using incubation times of 1 hour and 2 hours

Iproniazid (13 mg/ml) reduced the enzyme activity metabolizing noradrenaline in the spleen down to 55 % ($P < 0\,05$) within 2 hours and down to 3 % within 1 hour Even a concentration of 0 7 mg/ml inhibited noradrenaline inactivation in a very small extent

Amphetamine (7 mg ml) produced a 15 % decrease in the enzymatic inactivation of noradrenaline in bovine spleen tissue ($P < 0\,01$) A tenfold smaller concentration of amphetamine had no inhibitory effects on the metabolism of noradrenaline

When *potassium cyanide* was used the inactivation level was 89—98 % The inhibition was not significant

5 Lung

Bovine lung tissue (5 g) did not contain any clearly measurable amount of noradrenaline Accordingly noradrenaline (1 µg/g of tissue) was added to the homogenate before the incubation The control measurement gave 0 59—0 72 µg/g (recovery 59—72 %) as the noradrenaline amount The substrate concentration was thus 1 0 µM during the incubation After the 2 hour incubation noradrenaline was completely destroyed in two

different lung tissues and after the 1 hour incubation the amount of noradrenaline contained by the samples only produced a very slight blood pressure reaction

Enzyme Inhibitors

The studies with enzyme inhibitors have been made using 2 and 3 hours incubation times

Iproniazid (7 mg/ml and 13 mg/ml) reduced the noradrenaline metabolism to 71—40 % during the 3 hour incubation

At 0 7 mg/ml no decrease was produced During the 2 hour incubation the enzymatic inactivation of noradrenaline was inhibited by some 50 % at concentrations of 7—13 mg/ml whereas lower iproniazid concentrations had no clear inhibitory effects

The *amphetamine* induced inhibition of the enzymatic metabolism of noradrenaline was negligible at the concentrations used

Potassium cyanide produced no observable reduction in the inactivation of noradrenaline

6 Brain

The content of endogenous noradrenaline in bovine brain tissue was some 0 02 µg/g 0 5 or 1 µg of noradrenaline was added for every g of tissue before incubating the samples The noradrenaline content of the tissues to be incubated was thus 0 5 or 1 0 µM The recovery determined from corresponding control samples was on the average 0 30 µg/g or 0 53 µg/g (60 % or 53 %) As the average from three different tests 83 % and 98 % of noradrenaline were inactivated during the 2 and 3 hour incubation times respectively

Enzyme Inhibitors

Studies with enzyme inhibitors were made using incubation times of both 2 and 3 hours

Iproniazid (13 mg ml) reduced the enzyme activity down to 26 % of the maximum within the 2 hour incubation and down to 36 % of the maximum within the 3 hour incubation Even at 0 7 mg ml the enzyme activity of bovine brain tissue was 77 % and 78 % within corresponding incubation times

Amphetamine also produced a clear inhibition of noradrenaline inactivation Even at a concentration of 0 7 mg ml the amphetamine induced

inhibition was clearly observed in bovine brain tissue. In all the other tissues studied this amphetamine concentration had not inhibited the inactivation of noradrenaline significantly. At this concentration the enzymatic activity was 62 % of the maximum and 66 % of the maximum after the 2 and 3 hour incubation times respectively.

Potassium cyanide showed no noradrenaline metabolism inhibiting properties in bovine brain tissue.

7. Liver

Due to the small amount of endogenous noradrenaline it could not be measured in the 5 g liver tissue samples. Accordingly noradrenaline was added 0.40—1.0 μg/g of tissue. The noradrenaline concentration of tissue suspension to be incubated was thus either 0.4 or 1.0 μM. The recovery from corresponding control samples was, on the average, 0.20 μg/g or 0.55 μg/g (50 %—55 %). 84 % of liver tissue noradrenaline was inactivated within the 1 hour incubation and 100 % during the 2 hour incubation.

Enzyme Inhibitors

Studies with enzyme inhibitors were made using incubation times of both 1 and 2 hours. Noradrenaline inactivation was followed in liver homogenates from three animals altogether.

Iproniazid, as seen in table 8, effectively inhibited the inactivation of noradrenaline in liver at the high concentrations used. Even 0.7 mg/ml produced a slight inhibition.

Amphetamine (7 mg/ml) inhibited the noradrenaline inactivation to a significant degree ($P < 0.02$) within the two incubation times used. This inhibition was, however, of a smaller degree than that produced by the high concentrations of iproniazid. The enzyme activity of liver was not inhibited by a concentration of 0.7 mg/ml.

Potassium cyanide was not observed to have any inhibitory effects on the enzymatic inactivation of noradrenaline by liver tissue.

8. Kidney

The content of endogenous noradrenaline in bovine kidney tissue varied from 0.08 to 0.42 μg/g averaging 0.21 μg/g. Accordingly noradrenaline was added 1 μg/g of tissue. The exogenous noradrenaline concentration of the samples to be incubated was thus 1.0 μM. The recovery obtained in two simultaneous determinations with control samples was

79 % and 73 % respectively. Bovine kidney tissue from 3 animals altogether was studied. Noradrenaline was fully inactivated in kidney tissue homogenate within the 2 hour incubation and only a negligible amount (2 %) remained after the 1 hour incubation.

Enzyme Inhibitors

Studies with enzyme inhibitors were made using incubation times of both 1 hour and 2 hours.

Iproniazid, at its high concentrations (table 8) reduced the inactivation of noradrenaline in kidney tissue by about 50 % even after the 2 hour incubation. The result is statistically significant ($P < 0.01$). At the 0.7 mg/ml concentration iproniazid clearly inhibited noradrenaline inactivation during the 1 hour incubation but after 2 hours 92 % of the noradrenaline had been metabolized.

Amphetamine (7 mg/ml) inhibited the inactivation of noradrenaline but at 1/10 of the above concentration no inhibition was produced by amphetamine.

Potassium cyanide produced no clear inhibition of noradrenaline inactivation.

METABOLISM OF ADRENALINE IN GUINEA PIG TISSUE HOMOGENATES AFTER INJECTING MONOAMINE OXIDASE INHIBITORS *IN VIVO*

1. Method

In the aforegoing study the enzyme activity has been followed after adding the enzyme inhibitors to the tissue homogenates *in vitro*. Such conditions differ considerably from those in living organisms, e.g. the substrate and inhibitor concentrations may be very high. Consequently the monoamine oxidase inhibitors, iproniazid and amphetamine, were also injected into living organisms before the preparation and incubation of the tissues. Guinea pigs were used instead of bovine material. The results are not fully comparable with those obtained for bovine tissues, for the enzyme distribution and the enzyme induced metabolism differ from species to species (BAIN & BATTY 1956, SPECTOR *et al.* 1959, WEINER 1960, SHIDEMAN & GOLDBERG 1961). However, the results indicate the effect of these enzyme inhibitors in concentrations possible in living organism.

The enzyme activity was determined exactly in the same way as in the studies *in vitro*. Tissue homogenates were made from the guinea pig tissues to be studied. Adrenaline (or in some tissues noradrenaline) was added as substrate and the suspension was incubated at 37°C. The adrenaline metabolism rate in animals pretreated with enzyme inhibitors was compared with that in animals that had not been pretreated with the enzyme inhibitors.

Every test group included 3—4 male guinea pigs which were weighed and received the following enzyme inhibitors:

1st group 4 control guinea pigs, no inhibitors
2nd group 3 guinea pigs, iproniazid phosphate 20 mg/kg
3rd group 3 guinea pigs, iproniazid phosphate, 50 mg/kg
4th group 3 guinea pigs, amphetamine bitartrate 40 mg/kg

Two hours after the inhibitor injections the tissues to be studied were prepared and weighed. From this on the method was exactly the same as the one which used bovine tissues as the enzyme source and higher noradrenaline and adrenaline concentrations as substrates. The volume of the tissue homogenates was 5 ml, i.e. only half of the volume employed

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Every test group included 3—4 male guinea pigs which were weighed and received the following enzyme inhibitors:

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2nd group	3 guinea pigs	iproniazid phosphate, 20 mg/kg
3rd group	3 guinea pigs	iproniazid phosphate, 50 mg/kg
4th group	3 guinea pigs	amphetamine bitartrate 40 mg/kg

Two hours after the inhibitor injections, the tissues to be studied were prepared and weighed. From this on the method was exactly the same as the one which used bovine tissues as the enzyme source and higher nor adrenaline and adrenaline concentrations as substrates. The volume of the tissue homogenates was 5 ml, i.e. only half of the volume employed

in the first part. The tissue, however, comprised 10 % (w/v) of the suspension, the substrate concentrations of which were 0.1 mg/ml (2.96 and 2.73 µmol/5 ml) for noradrenaline and adrenaline respectively. Owing to the small amount of the tissues in this series (heart and spleen) both substrates could not be used in all the tests simultaneously. Most experiments have been made with adrenaline as the substrate. In the tissues where noradrenaline was also used as substrate (liver and kidney) the enzymatic metabolism of noradrenaline and its inhibition by enzyme inhibitors closely resembled those of adrenaline. Owing to the small volume to be used at the incubation the enzyme incubation was carried out in 20 ml test tubes. The blood buffer and indicators used when preparing the samples for the determinations were exactly the same as those described in the previous chapter when studying the enzymatic metabolism of noradrenaline and adrenaline in bovine tissue homogenates *in vitro*. The tissues of all the animals included in one test group were minced together and the rate of the enzymatic metabolism of adrenaline thus obtained represents the mean of the enzymatic activity present in the organs of 3—4 guinea pigs.

2. Results

Figures 15 and 16 present the enzymatic metabolism of adrenaline measured in guinea pig tissue after administering enzyme inhibitors *in vivo*. Each spot in the figure represents the amount of adrenaline metabolized within each time interval. The amount has been expressed as per cent of the original adrenaline amount and calculated using the optical density values representing the unmetabolized fraction of adrenaline after its oxidation to adrenochrome.

In guinea pig *liver tissue*, amphetamine (40 mg/kg) produced no clear inhibition of adrenaline metabolism (fig. 15). After one hour 81 % of adrenaline had been metabolized in the tissues of the controls, the corresponding value being 72 % for the amphetamine pretreated test animals. In the two iproniazid pretreated guinea pig groups (iproniazid 20 and 50 mg/kg) the inhibition of the adrenaline metabolism was quite clear: only 10 % of the adrenaline had been metabolized during the one hour incubation and oxidation. After the 4 hour incubation about half the amount of adrenaline had been destroyed in the liver of iproniazid pretreated guinea pigs.

In guinea pig *kidney tissue* (fig. 16) 92 % of the adrenaline had been metabolized in the controls within the one hour incubation and oxygenation. The corresponding metabolism for the amphetamine (40 mg/kg) pretreated group was about 70 %. After the 2 hour incubation with the enzyme

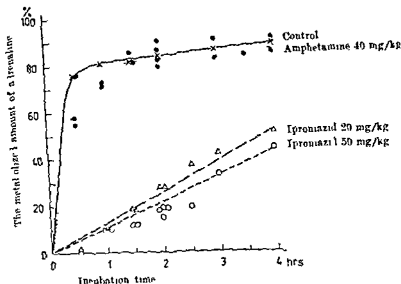


Figure 15

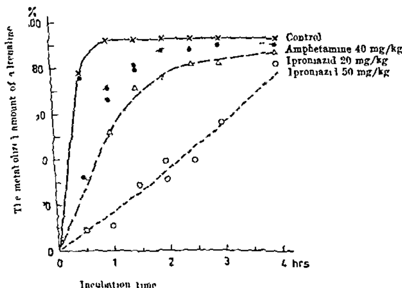


Figure 16

The effect of monoamine oxidase inhibitors in vivo on the metabolism of adrenaline in guinea pig kidney homogenate. Iproniazid and amphetamine have been injected i.p. 2 hours before the preparation of the tissues. [illegible]73 μmol of adrenaline has been added to 5 ml of 10% (w/v) kidney homogenate before incubation at 37°C.

in the first part The tissue however, comprised 10 % (w/v) of the suspension, the substrate concentrations of which were 0 1 mg/ml (2 96 and 2 73 μmol/5 ml) for noradrenaline and adrenaline respectively Owing to the small amount of the tissues in this series (heart and spleen) both substrates could not be used in all the tests simultaneously Most experiments have been made with adrenaline as the substrate In the tissues where noradrenaline was also used as substrate (liver and kidney) the enzymatic metabolism of noradrenaline and its inhibition by enzyme inhibitors closely resembled those of adrenaline Owing to the small volume to be used at the incubation the enzyme incubation was carried out in 20 ml test tubes The blood buffer and indicators used when preparing the samples for the determinations were exactly the same as those described in the previous chapter when studying the enzymatic metabolism of noradrenaline and adrenaline in bovine tissue homogenates *in vitro* The tissues of all the animals included in one test group were minced together and the rate of the enzymatic metabolism of adrenaline thus obtained represents the mean of the enzymatic activity present in the organs of 3—4 guinea pigs

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inhibitors the adrenaline metabolism in the amphetamine pretreated group was of the same extent as the metabolism in the control group of kidney tissues That the *inhibition* was dependent *on the amount of the inhibitor* injected was clearly seen in the guinea pig kidney, where some 50 % of the added adrenaline had been destroyed in the animals given the smaller iproniazid amount (20 mg/kg) whereas the corresponding metabolism was only 15 % in the kidney homogenate of animals given the larger iproniazid injection (fig 16)

In guinea pig *heart* and *spleen* tissues monoamine oxidase inhibitors at the concentrations used, produced no retardation in the metabolism rate of adrenaline (figs 17 and 18)

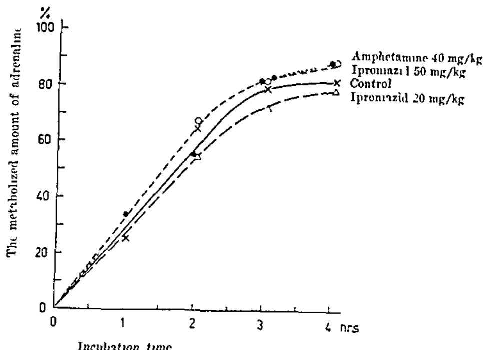


Figure 17
The effect of monoamine oxidase inhibitors *in vivo* on the metabolism of adrenaline in guinea pig *spleen* homogenate Iproniazid and amphetamine have been injected i p 2 hours before the preparation of the tissues 2 73 μmol of A has been added to 5 ml of 10 % (w/v) spleen homogenate before incubation at 37°C

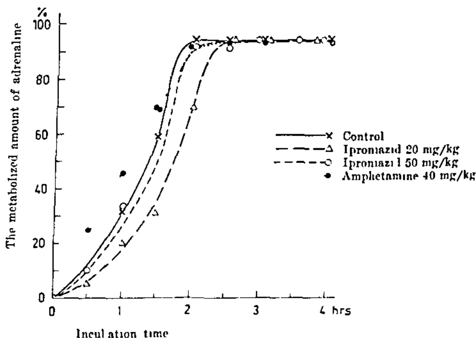


Figure 18
The effect of monoamine oxidase inhibitors *in vivo* on the metabolism of adrenaline in guinea pig *heart muscle* homogenate Iproniazid and amphetamine have been injected i p 2 *hours before* the preparation of the tissues 2 73 μmol of adrenaline has been added to 5 ml of 10 % (w/v) heart homogenate before incubation at 37°C

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In guinea pig *heart* and *spleen* tissues monoamine oxidase inhibitors at the concentrations used produced no retardation in the metabolism rate of adrenaline (figs 17 and 18)

DISCUSSION

The enzymatic metabolism of noradrenaline and adrenaline in bovine tissues *in vitro* has been studied by means of a developed chemical system for these enzyme studies (IISALO & PEKKARINEN 1958). In this method it was possible to apply the specific substrates present normally in the tissues, noradrenaline and adrenaline. In this respect the method differs from several earlier ones, e.g. manometric studies, where large amounts of tyramine has been used as substrate and the oxygen consumption measured. Since the noradrenaline and adrenaline contents had to be quite high (592—546 μM) to render photometric determinations possible in iodine oxidation reaction and to saturate the enzyme with substrate, studies were also carried out using the same tissues as enzyme sources but with the noradrenaline contents resembling those present in the organism (0.37—3.0 μM). In this case noradrenaline determinations were made biologically using the cat blood pressure method. Some studies were also made on the metabolism of adrenaline in guinea pig tissue homogenates *in vitro* following the administration of monoamine oxidase inhibitors *in vivo*. In the study the enzyme source was provided by tissue homogenates, in which the enzyme activity is usually lower than that in tissue slice preparations. The former are, however, more suitable for studies made with enzyme inhibitors.

Monoamine oxidase was observed to induce the metabolism of noradrenaline in all the tissues studied. The only exception was the skeletal muscle where the enzyme activity was very low. Even in earlier studies the low monoamine oxidase activity of the skeletal muscle has been observed (BHAGVAT *et al.* 1939, KOELLE & VALK 1954). The occurrence of monoamine oxidase in guinea pig diaphragm tissue has been observed by a histochemical method which makes use of the pigment formation from tryptamine (BLASCHKO & HELLMAN 1953). The highest enzyme activity was observed in liver, kidney and lung tissue. In all of them noradrenaline and adrenaline were completely destroyed within the two hour aerobic incubation under experimental conditions. The liver has been found to be a rich enzyme source in various animal species. Still there appear to be fairly large differences from species to species in this respect (BLASCHKO *et al.* 1937 a). E.g. in manometric studies rat liver, kidney and lung have been found to be equally effective in metabolizing adrenaline, although

the consumption of oxygen has been the smallest in the lung but still of the same magnitude as in the other organs (BLASCHKO *et al* 1937 a) In this study the metabolism of noradrenaline and adrenaline took place at similar rates in heart, uterus small intestine, spleen and brain At the higher substrate concentrations used the complete metabolism of these substances in these tissues lasted at least 4 hours under experimental conditions Metabolism of noradrenaline and adrenaline *in vitro* also took place in the heart, where the presence of monoamine oxidase activity has been observed by means of a histochemical method (KOELLE & VALK 1954)

In this study the enzymatic activity in one gram of tissue was in most cases capable of metabolizing the added one mg of noradrenaline or adrenaline within 4 hours In one liver suspension for which MICHAELIS's constant (K_m) was 1.7×10^{-3} M, 2 mg of noradrenaline was completely metabolized during the 2 hour oxidative incubation In experiments with higher substrate concentrations the enzyme was saturated with the substrate Reducing the substrate concentration to 1 300th—3000th of the original did not lead to a corresponding increase in the rate of the enzymatic metabolism of noradrenaline E g after the 1 hour incubation in an intestinal wall homogenate there still remained 0 21 μg/g of the original 2 2 μg/g noradrenaline Similarly after the 2 hour incubation in a heart muscle suspension there remained 0 09 μg/g from the original endogenous 0 41 μg/g noradrenaline Yet as regards these smaller, i e biological noradrenaline contents it is true that in most cases there was found no noradrenaline in the suspensions after the 1 hour incubation Studies using endogenous or fairly small noradrenaline contents of the tissues are although *in vitro* close to natural conditions

Noradrenaline and adrenaline were metabolized at the same rate in tissues under experimental conditions Accordingly tissues in which noradrenaline metabolism was rapid also exhibited a rapid adrenaline metabolism Noradrenaline and adrenaline being used simultaneously as substrates it has been observed that noradrenaline has become inactivated faster than adrenaline (BLASCHKO & BURN 1951, BURN & ROBINSON 1951, BAIN & BATTY 1952 1956) No significant difference between the metabolic rates was observed when noradrenaline and adrenaline were added simultaneously to heart muscle suspension *in vitro* (ITSALO & PEKKARINEN 1954 1958) ...

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The activity of monoamine oxidase depends on the oxygen pressure (KOHN 1937 PHILPOT 1937 BERNHEIM & BERNHEIM 1938) ...

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DISCUSSION

The enzymatic metabolism of noradrenaline and adrenaline in bovine tissues *in vitro* has been studied by means of a developed chemical system for these enzyme studies (IISALO & PEKKARINEN 1958). In this method it was possible to apply the specific substrates present normally in the tissues, noradrenaline and adrenaline. In this respect the method differs from several earlier ones, e.g. manometric studies where large amounts of tyramine has been used as substrate and the oxygen consumption measured. Since the noradrenaline and adrenaline contents had to be quite high (592—546 µM) to render photometric determinations possible in iodine oxidation reaction and to saturate the enzyme with substrate, studies were also carried out using the same tissues as enzyme sources but with the noradrenaline contents resembling those present in the organism (0.37—3.0 µM). In this case noradrenaline determinations were made biologically using the cat blood pressure method. Some studies were also made on the metabolism of adrenaline in guinea pig tissue homogenates *in vitro* following the administration of monoamine oxidase inhibitors *in vivo*. In the study the enzyme source was provided by tissue homogenates, in which the enzyme activity is usually lower than that in tissue slice preparations. The former are however more suitable for studies made with enzyme inhibitors.

Monoamine oxidase was observed to induce the metabolism of noradrenaline in all the tissues studied. The only exception was the skeletal muscle, where the enzyme activity was very low. Even in earlier studies the low monoamine oxidase activity of the skeletal muscle has been observed (BHAGVAT *et al.* 1939, KOELLE & VALK 1954). The occurrence of monoamine oxidase in guinea pig diaphragm tissue has been observed by a histochemical method which makes use of the pigment formation from tryptamine (BLASCHKO & HELLMAN 1953). The highest enzyme activity was observed in liver, kidney and lung tissue. In all of them noradrenaline and adrenaline were completely destroyed within the two hour aerobic incubation under experimental conditions. The liver has been found to be a rich enzyme source in various animal species. Still there appear to be fairly large differences from species to species in this respect (BLASCHKO *et al.* 1937 a). E.g. in manometric studies rat liver, kidney and lung have been found to be equally effective in metabolizing adrenaline although

homogenation facilitates the passage of iproniazid to the centre of the enzyme action (BRODIE & HOGBEN 1957)

A concentration of 1×10^{-3} M for potassium cyanide has been used with the intention of inhibiting cytochrome oxidase activity when studying monoamine oxidase in the Warburg system (e g BLASCHKO *et al* 1937 a ORZECHOWSKI 1941)

The iproniazid induced inhibition of the enzymatic metabolism of noradrenaline and adrenaline at high substrate concentrations using blood as buffer was observed to be of a smaller extent in most cases than the inhibition produced by amphetamine Yet at biological or near biological noradrenaline concentrations where the method made it possible to use higher inhibitor concentrations and where no blood buffer was used iproniazid was definitely more effective than amphetamine in inhibiting the metabolism of noradrenaline This phenomenon is partly explained by the finding which demonstrates that iproniazid loses its property to inhibit monoamine oxidase when incubated together with blood (ROEWER & WERLE 1957) In the chemical determination of noradrenaline and adrenaline iproniazid impeded the iodine oxidation reaction It consumed oxygen during the oxidation and when used in higher concentrations it thus prevented the formation of noradrenochrome and adrenochrome because of incomplete oxidation Iproniazid itself produced a slightly yellowish colour which disturbed the noradreno- and adrenochrome determinations It is possible that the oxidation of noradrenaline and adrenaline even at lower iproniazid concentrations could be somewhat incomplete and accordingly the iproniazid induced inhibition would not reach the expected level The higher iproniazid concentrations used in the biological determinations inhibited noradrenaline inactivation to a greater extent than did the concentrations used in connection with the chemical determinations The observation according to which the monoamine oxidase inhibition should require a threefold amount of iproniazid when the mitochondrial structure remains intact (AEBI 1961) has not been studied under experimental conditions

The metabolism of noradrenaline and adrenaline was clearly inhibited with the monoamine oxidase inhibitors in all the tissues where the chemical method was applied A hundred per cent inhibition was not obtained in any tissue Consequently it seems possible that there exist other enzymes taking part in the metabolism of these hormones or that there are other pathways for their destruction Catechol O methyltransferase requires the presence of S adenosylmethionine as a donator of the methyl group (AXELROD 1957)

A clear and statistically significant inhibition of the enzymatic metabolism of noradrenaline and adrenaline was produced by potassium cyanide

When neither an air nor an oxygen stream was used no metabolism of noradrenaline or adrenaline took place within 4 hours at 37°C (IISALO & PEKKARINEN 1958). Adding ascorbic acid as a reducing agent to the suspension during the incubation brought about a steep decrease in the oxidative metabolism of noradrenaline and adrenaline under experimental conditions (IISALO & PEKKARINEN 1958). During the incubation and oxygenation the samples were subjected to an even oxygen stream. This was achieved by connecting the test tubes in series of the same size. Still there may have been fluctuations in the oxygen stream as regards series which were oxygenated at different times. These variations may have resulted from differences in the consistence of the homogenates. Even the fact that the method depended on the temperature and pH supports the opinion of the enzymatic nature of the process studied.

As specific monoamine oxidase inhibitors the following were used: amphetamine sulphate (concentrations 7×10^{-4}—1.4×10^{-2} M) and iproniazid phosphate (concentrations 3.6×10^{-5}—1.8×10^{-3} M) at the chemical determinations and up to the concentration 4.7×10^{-2} M in the biological measurements. To inhibit the oxidation by cytochrome oxidase or some other heavy metal catalysis potassium cyanide was used (at concentrations 1.5×10^{-5}—1×10^{-4} M and adding it at one hour intervals to replace the probable decomposition of cyanide). Cytochrome c has not been added to the tissue suspensions to activate cytochrome oxidase. The specificity of the enzyme responsible for the cyanide sensitive destruction of noradrenaline and adrenaline has thus not been determined.

The specific ability of amphetamine to inhibit monoamine oxidase competitively *in vitro* was first observed by BLASCHKO (1940). The affinity of amphetamine to monoamine oxidase is high but the inhibition produced is reversible (PLETSCHER *et al* 1960). The limits of amphetamine concentrations used in different studies exceed those of the present study. In them amphetamine has been applied at concentrations 2.7×10^{-5}—2×10^{-2} M (e.g. ORZECHOWSKI 1941, BROWN & HEY 1956, IISALO & PEKKARINEN 1958). High amphetamine concentrations have been observed to inhibit cytochrome oxidase in addition to its monoamine oxidase inhibition (FRIEDENWALD & HERRMANN 1942). *In vivo* amphetamine has been shown to increase the rate of metabolism of noradrenaline and adrenaline (AXELROD & TOMCHICK 1959). Amphetamine prevents the uptake of circulating noradrenaline (AXELROD 1962) and releases the endogenous catecholamines from their stores (PAASONEN & VOGT 1956, BURN & RAND 1958).

The present iproniazid concentrations correspond with the concentrations generally used in experiments *in vitro* (e.g. ZELLER *et al* 1952, 1955, FURCHGOTT *et al* 1955, GRIESEMER & WELLS 1956, CORNE & GRAHAM 1957, SHORE *et al* 1957). The probable destruction of the cellular structure in

To find out the effect of the enzyme inhibitors when used in living organism the monoamine oxidase inhibitors amphetamine and iproniazid were injected into guinea pigs. The adrenaline metabolizing activity of their heart spleen kidney and liver tissue suspensions was compared with that of corresponding tissue homogenates made from the organs of control animals. Naturally these findings are not fully comparable to the results obtained with bovine tissues for there exist considerable differences between different animal species in the occurrence and activity of enzymes inactivating noradrenaline and adrenaline (SPECTOR *et al* 1959, WEINER 1960 SHIDEMAN & GOLDBERG 1961 CROUT *et al* 1961) Potassium cyanide could not be injected because of its toxicity In guinea pig liver, iproniazid (50 and 20 mg/kg) clearly reduced the adrenaline metabolism rate In kidney tissue, iproniazid (50 mg/kg) also proved to be an effective inhibitor In heart and spleen the injected enzyme inhibitor did not affect the adrenaline metabolism rate at any concentration and within the time chosen (2 hours after injection) Adrenaline metabolism was not inhibited by amphetamine at the concentration used (40 mg/kg) in any of the organs studied This is in agreement with the observation of AXELROD & TOMCHICK (1959) who could not show any inhibition of the metabolism of noradrenaline and adrenaline after amphetamine administration An increase in the excretion of noradrenaline and adrenaline after treatment with iproniazid has been found in guinea pigs the same species as used in the present study (PEKKARINEN *et al* 1960 b) The fact that adrenaline metabolism was retarded by iproniazid in guinea pig liver and kidney tissues supports earlier findings according to which monoamine oxidase lost its activity in different animal brain and liver tissues after iproniazid administration *in vivo* (ZELLER *et al* 1952 1955) It is also in agreement with the observation according to which the half life of adrenaline due to enzymatic metabolism was considerably lengthened in cat and guinea pig liver after iproniazid injections (GRIESEMER & WELLS 1956) In the whole rat, however the half life of injected adrenaline is not lengthened by β phenyl isopropylhydrazine or iproniazid which are both effective monoamine oxidase inhibitors (UDENFRIEND *et al* 1959 AXELROD & TOMCHICK 1960) Iproniazid is not fully able to penetrate into unbroken cells (BRODIE & HOGBEN 1957 UDENFRIEND *et al* 1957) Its distribution and penetration into organs may differ from tissue to tissue E g cerebral tissue is not easily penetrated by iproniazid (DAVISON 1958 b)

In the present study emphasis has been laid on the enzymatic metabolism of noradrenaline and adrenaline *in tissues* During the last few years a large number of studies have been made on the metabolism of exogenous and circulatory noradrenaline and adrenaline (e g AXELROD 1957, 1958, 1959 1960 AXELROD & LAROCHE 1959 AXELROD *et al* 1958 a 1958 b,

in bovine heart muscle in the present study when high noradrenaline and adrenaline concentrations were used. This inhibition was of the same magnitude as that produced by monoamine oxidase inhibitors in heart muscle. It has been demonstrated earlier that the inhibition of the metabolism of noradrenaline and adrenaline is greatly increased by the simultaneous addition of amphetamine and potassium cyanide to heart muscle suspension (IISALO & PEKKARINEN 1954, 1958). Consequently in bovine heart muscle *in vitro* there exists a cyanide sensitive enzyme system which is probably cytochrome oxidase. This result of the study supports the earlier finding according to which the heart muscle and skeletal muscle contain an adrenaline inactivating enzyme system which is sensitive to cyanide (PHILPOT & CANTONI 1941, HORNYKIEWICZ 1955). However, in addition to the cytochrome oxidase it seems that there is another cyanide sensitive heavy metal catalysis in rat heart muscle *in vitro* (HORNYKIEWICZ 1955). In the other bovine tissues studied the inhibition of the metabolism of noradrenaline and adrenaline by potassium cyanide was negligible.

In connection with biological or near biological noradrenaline contents the enzymatic activity of bovine tissues and the inhibitor induced reduction of noradrenaline metabolism were essentially similar to those observed in incubating considerably higher substrate concentrations in the same bovine tissues in connection with the chemical determinations. It is possible that the inhibitor induced reduction of the noradrenaline metabolism is rendered smaller by autoxidation during longer incubation periods used in the study. The monoamine oxidase inhibitor iproniazid prevented the metabolism of noradrenaline in all bovine tissues where the hormone was present at biological or near biological concentrations. A corresponding inhibition was also brought about by amphetamine in most tissues studied. Potassium cyanide did not reduce the metabolism of noradrenaline in any tissues when the noradrenaline concentration was low. The only exception was heart muscle where at low noradrenaline concentrations there was a similar inhibitory tendency as in connection with higher substrate concentrations. The inhibition in the heart muscle was, however, of a smaller extent and statistically significant in only one of the two different potassium cyanide concentrations used. Though it is true that the autoxidation of noradrenaline may occur in connection with this method and potassium cyanide may reduce autoxidation, it is still difficult to consider that the destruction of noradrenaline is reduced owing to this factor. A clear inhibition of the enzymatic metabolism was brought about by potassium cyanide only in heart muscle tissue when high noradrenaline and adrenaline concentrations were used. It seems more likely that bovine heart muscle contains a cyanide sensitive enzyme system which participates in the inactivation of endogenous tissue noradrenaline under experimental conditions.

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AXELROD & TOMCHICK 1960, KOPIN 1960, KIRSHNER *et al.* 1958, 1959, GOODALL *et al.* 1959). Of the two alternative enzymes inactivating noradrenaline and adrenaline *in vivo*, catechol-O-methyltransferase and monoamine oxidase, the former has been showed to play a more important role in inactivating exogenous *noradrenaline and adrenaline. Part of* methylated metabolites is then further deaminated by monoamine oxidase. Still most of the noradrenaline liberated at the nerve ends will get into tissues directly (VON EULER *et al.* 1954) where it may be inactivated through enzymatic action. Although it is known that exogenous radioactive nor*adrenaline taken up by cat spleen is, when liberated by electric stimulation,* mainly inactivated by catechol-O-methyltransferase (HERTTING & AXELROD 1961), there are several points which indicate that monoamine oxidase participates in the tissue metabolism of endogenous noradrenaline and adrenaline. Such are e.g. the increase in the amount of endogenous noradren*aline brought about by* structurally different monoamine oxidase inhibitors (PEKKARINEN *et al.* 1958, PLETSCHER 1958, SHORE *et al.* 1958, MUSCHOLL 1959 a, 1959 b, CARLSSON *et al.* 1959). Among them is also the prevention of the reserpine- or raunescine-induced noradrenaline release by monoamine oxidase inhibitors (e.g. SHORE *et al.* 1957, PEKKARINEN *et al.* 1958, PAASONEN & KÄRKI 1959, PLETSCHER 1959, PLETSCHER *et al.* 1959, WEIL-MALHERBE & BONE 1959, GREEN & SAWYER 1960). The release of nor*adrenaline by bretylium (PEKKARINEN et al. 1962) or by guanethidine* (KÄRKI, to be published) is also inhibited by monoamine oxidase inhibitors. Concerning these effects of monoamine oxidase inhibitors it is to be noted that the inhibition of the release of endogenous catecholamines may be more important than the retardation of the metabolism of these amines. The im*portance of monoamine oxidase in the tissue metabolism is also indicated by* the considerable change in the metabolism of injected radioactive noradren*aline in tissues brought about by monoamine oxidase inhibitors (KIRSHNER* 1960) and the principal part played by monoamine oxidase as the enzyme inactivating noradrenaline e.g. in rat heart and brain tissue (CROUT *et al.* *1961). The observations made in the present study on the metabolism of* noradrenaline and adrenaline in bovine tissues, though made *in vitro* and as such not fully applicable to conditions *in vivo*, also support the part played by monoamine oxidase in the tissue metabolism of noradrenaline and adrenaline.

However, only *small differences in the rate or in the direction of* the metabolism of drugs can be produced by enzyme inhibitors. The organism is effective enough to metabolize drugs in spite of the inhibition of one enzyme system and it is capable of finding new metabolic pathways. Studies on the metabolism of endogenous hormones in tissues are needed to ascertain *definitely the part played by various enzyme* systems in metabolic processes in the organism.

SUMMARY

The enzymatic metabolism of noradrenaline and adrenaline *in vitro* has been followed in homogenates made from bovine heart, skeletal muscle, lung, uterus, small intestine, spleen, liver, kidney and brain tissues. The method for this study has been described earlier (IISALO & PEKKARINEN 1958). The enzyme incubation was performed at pH 7.4 at 37°C in a continuous oxygen stream. The unmetabolized fraction of noradrenaline and adrenaline was determined after the incubation. Noradrenaline and adrenaline were used as the specific substrates in the tissue homogenates. Their concentrations were 592 µM and 546 µM respectively, at which concentrations the enzyme was saturated with substrate. Chemical determination of noradrenaline and adrenaline was performed converting them by iodine oxidation reaction to noradrenochrome and adrenochrome which were determined photometrically. Noradrenaline metabolism was also followed at biological or near biological concentrations in the same bovine tissues (0.37—3.0 µM) using a biological determination.

Amphetamine sulphate and iproniazid phosphate were used as specific monoamine oxidase inhibitors. Amphetamine (at concentration 7×10^{-4}—1.4×10^{-2} M) and iproniazid (at concentration 3.6×10^{-5}—4.7×10^{-2} M) were added to the tissue homogenates before the incubation. With the intention of inhibiting the possible oxidation due to cytochrome oxidase, potassium cyanide (at concentration 1.5×10^{-5}—1×10^{-4} M, added at one hour intervals) was used.

The enzymatic metabolism of noradrenaline and adrenaline was observed to take place in all the bovine tissues studied. The only exception was skeletal muscle in which the metabolism of noradrenaline and adrenaline was very small after the 4 hour incubation and oxygenation.

The greatest noradrenaline and adrenaline metabolism rate as regards the high substrate concentrations was observed in liver, kidney and lung homogenates. In all of them more than half of the substrate had been metabolized within the one hour incubation and oxygenation under experimental conditions. In heart, uterus, small intestine, spleen and brain tissue suspensions the metabolism of the high noradrenaline and adrenaline concentrations was of the same magnitude and requiring at least a 4 hour oxidative incubation for complete metabolism under experimental conditions. The noradrenaline metabolism rate at biological or near biological concentrations was considerable greater than that observed

at the high concentrations. Yet it was not proportional to the 300—3000 fold decrease in the substrate concentration.

Monoamine oxidase inhibitors amphetamine and iproniazid produced a clear inhibition of noradrenaline and adrenaline metabolism in all the *bovine tissues where enzymatic metabolism had occurred.* Almost similar inhibition was brought about at the biological or near biological concentrations. Yet the inhibition of noradrenaline metabolism produced by *amphetamine remained below that observed at the higher substrate* concentrations.

A hundred per cent inhibition of noradrenaline and adrenaline meta*bolism* was produced by monoamine oxidase inhibitors in no tissue. Thus it seems likely that in addition to monoamine oxidase there exists other enzyme systems participating in the metabolism of noradrenaline and adrenaline in bovine tissues.

At least *in vitro* monoamine oxidase participates in the enzymatic metabolism of a) added large noradrenaline and adrenaline contents in bovine heart, lung, uterus, small intestine, spleen, kidney, liver and brain, b) small endogenous noradrenaline contents in bovine heart and spleen and c) near biological contents of added noradrenaline in all the above mentioned bovine tissues.

Potassium cyanide did not produce a statistically significant inhibition of the metabolism of large amounts of exogenous noradrenaline and adrenaline or *the metabolism of biological amounts of noradrenaline when* incubated in bovine lung, uterus, small intestine, spleen, kidney, liver or brain tissue suspensions. Yet the enzymatic metabolism of large added amounts of *noradrenaline and adrenaline was significantly inhibited by* potassium cyanide in bovine heart tissue homogenate. The metabolism of small endogenous amounts of noradrenaline in bovine heart tissue was also *inhibited by potassium cyanide. The inhibition was of a small extent but nevertheless significant. Consequently bovine heart muscle tissue in vitro* contains a noradrenaline and adrenaline metabolizing enzyme system which is *sensitive to potassium cyanide. It is probably cytochrome oxidase. However* the specificity of this enzyme system has not been studied in the present investigation.

Monoamine oxidase *inhibitor iproniazid (20—50 mg/kg)* when injected into living guinea pigs produced a clear inhibition of the adrenaline metabolism in liver and kidney homogenates when incubated *in vitro*. Whereas *iproniazid in vivo* induced no effect on the metabolism of adrenaline in heart and spleen tissue homogenates. Monoamine oxidase inhibitor amphetamine when administered to living guinea pigs at the concentration of *40 mg/kg* did not inhibit the enzymatic metabolism of adrenaline taking place in heart, liver, kidney and spleen tissue homogenates.

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CONTENTS

INTRODUCTION

Rare earth metals do not appear so sparsely in nature as the name implies Many of them are equally as common as other, better known elements The fact that interest in rare earth metals was awakened rather late probably, among other reasons depends upon the great technical difficulties in isolating them Since these problems have been solved, the metals have become more and more of current interest within industry and medicine When atomic energy began to be used in nuclear weapons and reactors, the development of rare earth metals moved further forward Since the last world war, many new perspectives have been opened with the help of these elements

The significance of the rare earth metals can, from a biological point of view, be brought together into the following three points, namely

1 as toxic elements
2 as helpful agents in medicine
3 as fission products

It can thus be clearly seen that it is of importance to be familiar with the rare earth metals behavior in and their influence on the organism In their metabolism the liver and the skeleton play a dominating role The aim of the present investigation is to elucidate some aspects of the behavior of rare earth metals The following problems will be taken up for treatment

1) The uptake of rare earth metals by the liver and the kidneys and their excretion via the urine the bile, and the gastro intestinal tract,
2) The subcellular distribution of the rare earth metals in liver cells,
3) The acute toxic effects of the rare earth metals on the liver

The chemical similarities between the rare earth metals are well known There is also reason for believing that the actinides have a lot in common with at least some of the lanthanons. Due to these similarities, experiments are being done in order to carry over the properties of one metal to another in spite of the fact that an experimental basis for this is missing An attempt has been made here to obtain a conception of the entire group of rare earth metals As representative cerium promethium, terbium, holmium, and ytterbium were chosen Of these cerium is found in the beginning and ytterbium in the end

of the lanthanon series while those remaining are placed between these two elements In addition, yttrium was investigated since, because of its great similarity to the rare earth metals, it is often treated along with them Yttrium also is an important metal from a medical and fall out point of view One may recall that yttrium-90 is a daughter nuclide of strontium-90

CHAPTER I

Literature Survey

In the periodic system, the rare earth metals are placed in group no III, and with them, elements with atomic numbers of 57—71 are included Yttrium and sometimes even scandium are classified with these elements because of their great similarity to the rare earth metals The first metal which belongs to the rare earth metals is lanthanum From here on, the terms rare earth metals and lanthanons will be used interchangeably Likewise yttrium will be regarded as a lanthanon in this work Lanthanons are usually divided into light and heavy To the former belong lanthanum, cerium, praseodymium, neodymium, promethium samarium, europium, and gadolinium, and, to the latter terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium Occasionally, reports divide them into three groups with a middle or transitional group which includes europium, gadolinium, and terbium

The chemistry of the rare earth metals is treated in a number of works As examples of these the surveys of Vickery (1953, 1960) and O Brien et al 1955) can be named The discovery of the rare earth metals and many investigators toilsome and interesting work in this connection has been treated in detail by Weeks (1948) These cited authors lay a foundation for much data on the rare earth metals The biological and pharmacological properties of the rare earth metals are handled in a detailed survey by Steidle (1935) Their significance in biochemical and medical research has been illuminated by Kyker & Andersson (1956) An extensive literature survey of yttrium has been done by Ramsden (1961) A summary of the whole body of lanthanons has been given by Kyker (1962) Since these references cover the work on rare earth metals so well no detailed literature survey will be presented here

The Discovery of the Rare Earth Metals

The history of the rare earth metals begins with 'yttria" and "ceria", which consist of oxides Completely erroneously, they were, in the beginning of their discovery regarded as pure oxides "Yttria" was discovered by the Finnish chemist Johan Gadolin in 1794 in a mineral found 6 years earlier at Ytterby, outside of Stockholm Some years later, 1803, Klaproth, Berzelius, and Hisinger completely independent of each other, discovered "ceria' After this

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TABLE I The Lanthanons — their discoverers and the derivation of their names (O'Brien et al 1955)

Name	Symbol	At No	Discoverer	Date	Derivation of Name
Cerium	Ce	58	Berzelius	1814	Planet Ceres
Dysprosium	Dy	66	Boisbaudran	1886	Greek meaning hard to get at
Erbium	Er	68	Berlin	1860	Ytterby, a town in Sweden
Europium	Eu	63	Demarcay	1896	Europe
Gadolinium	Gd	64	Marignac	1886	Finnish chemist Gadolin
Holmium	Ho	67	Cleve	1879	Latin *Holmia* for Stockholm
Lanthanum	La	57	Mosander	1837	Greek, *lanthano* meaning to conceal
Lutetium	Lu	71	Urbain and Welsbach	1907	*Lutetia* ancient name for Paris
Neodymium	Nd	60	Welsbach	1885	Greek *neos* "new and *didymos* twin
Praseodymium	Pr	59	Welsbach	1885	Greek *praseo* green and *didymos* twin"
Promethium	Pm	61	Marinsky, Glendenin and Coryell	1947	Greek Prometheus
Samarium	Sm	62	Boisbaudran	1879	Samarski a Russian army officer
Scandium	Sc	21	Nilson	1879	Scandinavia
Terbium	Tb	65	Boisbaudran	1895	Ytterby a town in Sweden
Thulium	Tm	69	Cleve	1879	Thule "northland"
Ytterbium	Yb	70	Urbain and Welsbach	1907–8	Ytterby a town in Sweden
Yttrium	Y	39	Mosander	1842	Ytterby a town in Sweden

beginning a sequence of attempts to split yttria and ceria followed Because of the lanthanons' chemical similarities and their blended minerals it was extremely difficult to separate them Separation techniques were developed however, and soon one element after another was crystallized out from yttria and ceria' It was thus shown that yttria was a combination of oxides of yttrium terbium erbium ytterbium lutetium holmium thulium and dysprosium Ceria' consisted of oxides of cerium lanthanum neodymium praseodymium samarium gadolinium europium and promethium Table I shows the discoverers and the derivations of the names of the rare earth metals

The Natural Occurrence of Lanthanons

The rare earth metals occur in the earth's crust (Table II) where they are as common as some of the better known elements such as lead tin zinc arsenic mercury, gold and platinum Fig 1 shows the occurrence of the lanthanons in

Table II Relative abundance of rare earth metals (O Brien et al 1955)

Rare Earth		Percentage of Group	Percentage of Earth s Crust	Gram/ton of Earth s Crust
Y	39	—	—	31 0
La	57	7	0 00035	19 0
Ce	58	31	0 00155	44 0
Pr	59	5	0 00025	5 6
Nd	60	18	0 00090	24 0
Pm	61	0	0 00000	—
Sm	62	7	0 00035	6 5
Eu	63	0 2	0 00001	1 0
Gd	64	7	0 00035	6 3
Tb	65	1	0 00005	1 0
Dy	66	7	0 00035	4 3
Ho	67	1	0 00005	1 [illegible]
Er	68	6	0 00030	2 4
Tm	69	1	0 00005	0 3
Yb	70	7	0 00035	2 6
Lu	71	1 5	0 00007	0 7

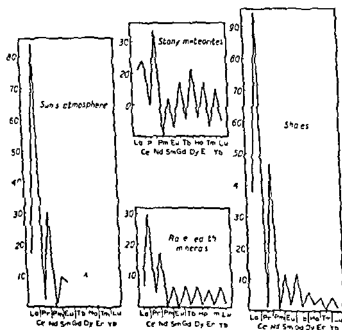


Figure 1 Atomic abundance of the lanthanons (Vickery 1953)

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Europium	Eu	63	Demarcay	1896	Europe
Gadolinium	Gd	64	Marignac	1886	Finnish chemist, Gadolin
Holmium	Ho	67	Cleve	1879	Latin, *Holmia*, for Stockholm
Lanthanum	La	57	Mosander	1837	Greek, *lanthano*, meaning to conceal
Lutetium	Lu	71	Urbain and Welsbach	1907	*Lutetia*, ancient name for Paris
Neodymium	Nd	60	Welsbach	1885	Greek, *neos* new and *didymos* twin
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earths, while the least basic, scandium is comparable to aluminum The solubility of the oxides increases with increasing atomic number In general, the chlorides, nitrates bromides, and iodides are highly soluble in water The sulfates are moderately soluble The oxides hydroxides, phosphates carbonates, and oxalates are difficult to dissolve in water

The Significance of the Lanthanons

One of the reasons that the rare earth metals lacked practical interest in the past was the great difficulty in preparing the various elements in their pure forms Today, the rare earth metals can be obtained with a high degree of purity, and therewith, many areas of use have come about Not the least of these being industry which has shown its interest in rare earth metals

a) *Within industry*

Lanthanons are used in the ceramic and glass industries There are lanthanon containing glass filters which completely absorb ultra violet and infrared rays Lanthanons occur also in optical glass The lanthanons play an important role in various alloys along with other metals In alloys, one often proceeds from "Mischmetall" which is a mixture of Ce, La, Pr, and Nd with a proportion of 70 per cent cerium Cerium is the most important and most used lanthanon in industry Generally it is an alloy of iron, but is also found combined with Ni Co, Sn, Zn, Mg, Nb, Cr, Mo, and W As an example, cigarette lighter flints can be named which consist of 70 per cent Ce and 30 per cent Fe Lanthanons are further used in gas mantles coal arc lights, signal lights, and electrodes In the chemical industry, lanthanons are used as catalysts

Industrial use of the lanthanons, which in the future will probably increase brings with it biological problems of a hygienic character In working with lanthanons the air can contain air borne metal particles which can be inhaled Wounds are also a conceivable means of entrance into the body Hereby, the value of knowing the toxic effect that the lanthanons may have on the organism can be seen This has been emphasized by among others Haley et al (1961) Chen et al (1961), and Kyker (1962)

b *Within medicine*

Many of the characteristic biological actions of the rare earth metals were observed rather early (Steidle 1935) Some of the lanthanons were also used in medical therapy, for example, as antiseptics anti motion sickness drugs, and as anti tuberculosis agents Nowadays, the lanthanons are being used therapeutically in two regions namely, as anti thrombotic agents, and in the treatment of certain tumors

TABLE III Some physical properties of the lanthanons (O Brien et al 1955)

Element	Atomic Number	Atomic Weight	Density	Melting Point (°C)	Boiling Point (°C)	Atomic Radius (A)	Color of Salts of M^{+3}
Scandium	21	44 96				1 87	Colorless
Yttrium	39	88 92	5 57	1490	2500		Colorless
Lanthanum	57	138 92	6 14	825±5	4242±150	1 87	Colorless
Cerium	58	140 13	6 80	675		1 81	Colorless
Praseodymium	59	140 92	6 8	535	3017±90	1 82	Green
Neodymium	60	144 27	7 0	840		1 82	Red
Promethium	61	145					
Samarium	62	150 43	6 93	1300			Pink
Europium	63	152 0	5 24			2 04	Rose
Gadolinium	64	156 9	7 94			1 79	Colorless
Terbium	65	159 2	8 33			1 77	Colorless
Dysprosium	*66*	*162 46*	*8 56*			*1 77*	*Yellow*
Holmium	67	164 94	8 76			1 76	Yellow
Erbium	68	167 2	9 16			1 75	Red
Thulium	69	169 4	9 35			1 74	Green
Ytterbium	70	173 04	7 01			1 93	Colorless
Lutetium	71	174 99	9 74			1 74	Colorless

various locations It should be pointed out that elements with even atomic numbers occur in larger amounts than elements with odd atomic numbers The same rule also applies for other elements (Harkin's rule) The largest deposits of the lanthanons occur in Brazil, India, the Ural Mountains, Scandinavia and the U S A Today, man is familiar with about 150 different minerals which consist of lanthanons

Physical and Chemical Properties of the Lanthanons

A collection of some of the physical properties is found in Table III In connection with this, it should be observed that the atomic radius usually diminishes with increasing atomic numbers This is called the contraction of lanthanons This behavior is sometimes discussed in a biological connection (Durbin 1962)

The metals are easily dissolved in dilute mineral acids However, cerium is dissolved only slowly in dilute nitric acid As a rule the lanthanons form trivalent ions but bivalent as well as *tetravalent ions exist* Concerning chemical compounds of lanthanons, the follwing may be noted from a biological view point The hydroxides are difficult to put into solution and are insoluble in an excess of alkali The oxides are rather strong bases The alkalinity diminishes as atomic numbers increase with the exception of yttrium and scandium Lanthanum which is the most basic, is, in this reference, *comparable to alkaline*

TABLE IV Fission products important on account of yield and half life (Bolles & Ballou 1956) (Atomic yield > 0.03 per cent, half life > 10 hours)

Chain atomic weight	Fission yield atom per cent	Nuclide and half life	
85	1.3	Kr (10 y)	
89	4.6	Sr (51 d)	
90	5.1	Sr (28 y)	→ Y (61 h)
91	5.4	Sr (9.7 h)	→ Y (57 d)
93	6.0	Y (10 h)	
95	6.3	Zr (65 d)	→ Nb (35 d)
97	6.4	Zr (17 h)	→ Nb (74 m)
99	6.0	Mo (68 h)	→ Tc (5.9 h)
103	3.4	Ru (40 d)	→ Rh (57 m)
105	1.0	Ru (4.5 h)	→ Rh (36 h)
106	0.5	Ru (1.0 y)	→ Rh (30 s)
127	0.16	Sb (93 h)	→ Te (90 d 20 % 9.3 h 80 %)
129	0.9	Te (32 d 21 %)	
131	3.1	I (8.1 d)	
132	4.0	Te (78 h)	→ I (2.4 h)
133	6.3	I (22 h)	→ Xe (5.3 d)
135	6.0	I (6.7 h)	→ Xe (9.2 h)
137	6.2	Cs (37 y)	→ Ba (2.6 m)
140	6.1	Ba (12.8 d)	→ La (40 h)
141	6.0	Ce (33 d)	
143	5.0	Ce (33 h)	→ Pr (13.7 d)
144	5.0	Ce (290 d)	→ Pr (17.5 m)
147	2.9	Nd (11.6 d)	→ Pm (3.7 y)
149	1.4	Pm (47 h)	
151	0.5	Pm (27.5 h)	→ Sm (73 y)
153	0.16	Sm (47 h)	
155	0.03	Eu (1.7 y)	

radioisotopes of yttrium (Kyker 1962) are formed. The most important fission products are stated in Table IV. The rare earth metals account for a not unessential part of the fission products. They dominate, in this manner, between the 70th day and six years after the fission of uranium 235 (Low & Bjornerstedt 1957). Cerium 144 is the radionuclide which appears in the highest amount among these products from one to four years after fission (Liebscher et al. 1961).

The biological dangers of fission products are nowadays generally known. Knowledge of the behavior of radionuclides in the living organism plays an important part in the defense against this potential danger. This problem has aspects in food and industrial hygiene.

The rare earth metals have a retarding effect on blood coagulation (Steidle 1935, Vincke & Oelkers 1937, Beaser et al 1942, Hara & Sato 1955, author's own observations) *This property of the lanthanons led to exploration for a* non-toxic lanthanon compound which could be utilized as an anti-thrombotic agent (Vincke 1955) For this purpose, a neodymium salt of 3-sulfo-isonicotinic acid was shown to be best This salt is found in Thrombodym® whose action and practical use is described by Laas & Vincke (1957) and by Vincke (1960) According to some investigations (Hara & Sato 1955), the lanthanons were thought to have their anti-coagulant effect by inhibition of prothrombin synthesis Other investigations (Hunter & Walker 1956 a, 1956 b) show, however, that the anti coagulant effect is due to action on the Christmas factor and factor X

For irradiation of tumors, the question of suitable radioisotopes arises One third of the radioisotopes which are used *for medical therapy are lanthanons* (Brucer 1952) Lanthanon isotopes are particularly adapted for irradiation of tumors in serous cavities from which they are poorly resorbed (Laszlo et al 1952, Spode 1958, Spode & Gensicke 1961)

Due to its half-life (61 hours) and radiation energy (E_{max}2 24 MeV), yttrium-90 has perhaps been the foremost one to come into use for this purpose An inhibiting effect on the growth of ascites tumors in mice has been obtained by lanthanum-140 (Lewin et al 1953) and yttrium-90 (Lewin et al 1954 b) Lately, patients have been treated with Y^{90} intraperitoneally with a beneficial *effect In a work by Siegel et al (1956), the value of palliative treatment in* man with Y^{90} has been pointed out His patients had cancer with pleural and peritoneal effusions A comparison between Y^{90} and Au^{198} after intravenous administration shows that Y^{90} produces the least damage to bone marrow (Scheer 1956) Certain hematologic disturbances such as multiple myeloma and polycythemia vera have been treated with Y^{90} intravenously with encouraging results (Sawitsky et al 1960, Greenberg et al 1962) Europium 152 *and -154 have also been used for irradiation therapy (Meschan et al 1956)* Among the rare earth metals are many others, e g La^{140}, Ho^{166}, Yb^{169}, Yb^{175} and Lu^{177}, with potential value for similar irradiation (Kyker 1954 Christo*phersson et al 1956)*

c) *As fission products*

The occurrence of rare earth metals among fission products furnishes a *motive* for observation from a biological viewpoint About 200 nuclides of some 60 elements are formed after fission of uranium-235 (Behrens 1959) Approximately 85 per cent of *these radioactive* isotopes have half lives ranging from a few seconds to a great many years Radioactive fission products are formed both in reactors and in nuclear weapons At least ten radiolanthanons and six

The Action of pH and Carrier on the Distribution of the Lanthanons

The pH of the injected solution influences the distribution picture (Spode & Gensicke 1961, Aeberhardt et al 1961) With subcutaneous and intraperitoneal injection of $CeCl_3$ at various pH to mice, Spode & Gensicke (1961) obtained a maximal uptake in the liver, spleen, kidneys, and femur when the pH was 3 5 Aeberhardt et al (1961) gave $CeCl_3$, pH 4 and 9, intravenously to rats The investigations showed with the lower pH a lower uptake in the liver and spleen than with the higher pH, while in relation to the skeleton and kidneys, the opposite occurred

Investigations show that addition of carrier to the injection solution changes the distribution picture Lewin et al. (1954 a) obtained a lowered resorption of radioyttrium from the abdominal cavity when carrier was added Carrier addition also reduces the resorption of yttrium after subcutaneous injection (Gensicke & Spode 1961) The same authors also showed that with intravenous injection, carrier changes the distribution of yttrium so that there is a greater uptake in the liver and less in the skeleton During radiation treatment of tumors in serous cavities, the aspired poor resorption can consequently be attained through the addition of carrier

Radiocolloid Formation

In low concentrations, the rare earth metals can form colloids Thus, in solutions, their radioisotopes can form radiocolloids The theory of their appearance and the problems in connection with the formation of radiocolloids have been treated by, among others Schweitzer & Jackson (1952) The appearance of radiocolloids is influenced by, among other things, the pH of the solution The physical-chemical condition in solutions of radiocerium has been studied by Aeberhardt (1961) and Aeberhardt et al (1962) According to these investigations $Ce(NO_3)_3$ is completely ionized at a pH under 3 and $CeCl_3$ with a pH under 4 5 With a pH over 11.5, cerium always occurs in colloidal form irrespective of the anions present An equilibrium between ionized and colloidal cerium always occurs at a certain pH It is possible that a similar condition pertains also to the other *lanthanons*

The Toxicity of Lanthanons

Industrial use of lanthanons might cause hygienic problems. The literature is, however, rather sparse on this subject Kyker & Cress (1957) reviewed the literature and performed a toxicological investigation. The toxic effects of gadolinium and samarium have recently been studied by Haley et al (1961)

Metabolism of the Lanthanons in the Organism

The metabolism of the rare earth metals in small animals (mice, rats, guinea pigs) has been studied in a succession of works (Hamilton 1948, 1949, Durbin et al 1956, Spode 1958, Graul & Hundeshagen 1959, Durbin 1960, Spode & Gensicke 1960, 1961, Gensicke & Spode 1961, Aeberhardt 1961, Rosoff et al 1963) Liver and bone tissue play a dominating role but the kidney and spleen also have a not unessential place in this connection

The distribution of the lanthanons is, to a great extent, dependent upon the method of administration Experimental investigations show that oral administration produces a very insignificant resorption from the gastro intestinal tract This does not only apply to small animals but pertains also to ruminants (Garner et al 1960, Ekman & Åberg 1961)

From a practical viewpoint, perhaps the most important entry into the body of radionuclides which are only insignificantly resorbed through the gastrointestinal tract is the aerogenous route Following radioactive fallout, radio active elements appear in the air and can be carried into the body by inhala tion There are results which indicate that inhalation corresponds rather well to intravenous administration (Norris et al 1956) Depending upon size and solubility, the inhaled particles can be resorbed, fixed in the lungs, carried via the lymph and taken up by lymph nodes, or carried up to the mouth and then swallowed (Schubert 1951, Stannard 1959) An uptake in the pulmonary lymph nodes after inhalation has been observed in man (Liebscher et al 1961)

Intraperitoneal administration is often practiced in experimental investigations and in tumor therapy As was earlier intimated, the lanthanons have a very poor resorption from serous cavities This is a favorable condition for irradiation of tumors located here

With subcutaneous and intramuscular injections, varying resorption conditions exist (Kyker 1962) Soluble lanthanon complexes (for example the citrate complex) are resorbed moderately well (Durbin et al 1956) However, lanthanon compounds, which at the site of injection form insoluble com pounds, are poorly resorbed At the same time, however, they are transported to the regional lymph nodes (Christophersson et al 1956) Intravenous administration of lanthanons is often used in experimental studies and sometimes in therapeutic irradiation Lanthanons in blood are quickly taken up by the liver A short time after administration, this organ contains, generally, more than 50 per cent of the given dose The skeleton also accounts for a large uptake, often more than 20 per cent The remaining organs and tissues contain considerably less of the lanthanons which are excreted via feces as well as urine Cerium is also excreted via bile (Castellino et al 1962)

are bound to albumin *in vitro* as well as *in vivo* (Ekman et al 1961) This complex formation was demonstrable as early as five minutes after the elements had been administered intravenously Levin (1954) also reported that yttrium is attached to serum proteins She was able to demonstrate that complex formation is a reversible reaction Aeberhardt et al (1961) reported that ionized cerium *in vivo* as well as *in vitro* is bound to γ- and β_1-globulins Their investigations were, however, performed with a paper electrophoretic technique and a complex formation between the cellulose of the paper and the rare earth metals is probable Kyker et al (1955, 1957 b) found in dogs that half of an intravenous dose of Lu^{III}, Ho^{III}, and Nd^{III} was bound to the globulin fraction

According to the literature cited here, the nature of the protein attachment of lanthanons as well as the different behavior towards the serum proteins is controversial The explanation most probably lies in the various experimental conditions used There is, however, no doubt that ionized lanthanons in blood are transferred to non ionized complexes Non ionized colloids are transported as such according to Aeberhardt et al (1961)

In the metabolism of lanthanons within the cells, the behavior towards nucleic acids is of interest Trivalent ions, among them lanthanum, were reported (Hammarsten 1924) as precipitating nucleic acids and lanthanum was used for this by Hammarsten and Teorell (1928) In analytical work, the lanthanum nucleic acid complex was used by Caspersson and co-workers (Caspersson et al 1935, Caspersson 1936) and by Opie & Lavin (1946) Stern & Steinberg (1953) reported that Pr, Nd, Sm, and Y also precipitate nucleic acids Serum phosphatides show a marked affinity for yttrium (Graul & Hundeshagen 1959)

An influence of lanthanons on phosphatases has been reported by Bamann (1954) and Bamann et al (1954 a, 1954 b) This pertains to phosphate-containing compounds which are steps in the general metabolism such as hexosephosphates, ADP ATP, RNA, and DNA It is probable that the influence of lanthanons on the cells of the animal organism is, to a large part, due to this interference with phosphate transport Trapmann (1959) reviewed the catalytic properties of lanthanons

Lanthanons will, as will aluminum, accelerate the oxygen uptake in a succinic dehydrogenase cytochrome oxidase system (Horecker et al 1939) Sections from cerium induced fatty liver show a lower uptake of oxygen than normal liver sections (Kyker et al 1957 a) Glenn et al (1962) reported that cerium-induced fatty liver lacks the ability to oxidize fatty acids and that it also shows manifest inhibition of oxidative phosphorylation Together with a marked ATP ase activity increase it was taken as a sign of serious mitochondrial injury in the liver cells The lowered capacity for fatty livers to oxidize fatty acids has also been shown by Snyder et al (1960 b)

who also reported work on the heavy lanthanons (1962) When locally applied, a number of the lanthanons cause persistent corneal injury (Grant & Kern 1956) The route of administration will greatly influence the toxic effects on the organism, toxicity being proportional to the amount taken up by the organism At toxic levels, necrosis will occur locally at the injection site after intramuscular and subcutaneous administration (Graca et al 1957) Peritonitis was reported by Steffee (1959) following intraperitoneal injections

As already pointed out, the liver plays a dominant role in the metabolism of lanthanons This was noticed as early as 1938 by Fischler & Roeckl The great interest in lanthanons during the last decades has caused a number of investigations of liver damage This damage consists of fatty changes of the liver but only the light lanthanons (up to and including samarium) will produce it (Kyker et al 1957 a, Snyder et al 1959, Neubert & Hoffmeister 1960, Snyder et al 1960 a, Snyder & Stephens 1961, Glenn et al 1962) Fatty liver, however, can only be produced under certain conditions This problem has been studied chiefly in rats where it was shown that fatty liver occurred only in the female This has been explained by hormonal influence (Snyder et al 1959)

Biochemical Properties of Lanthanons

Lanthanons have no known function in the mammalian organism Much work was done earlier in order to try to determine the normal content of lanthanons in various organs Small amounts have been found mainly in the skeleton and the kidneys It is impossible, however, from these results to deduct if they have any physiological role (Kyker 1962) Because of their pharmacological effects (Steidle 1935), their biochemical behavior is of interest This interest was enhanced when it was found that lanthanons induce fatty liver

It can be assumed that, in the blood, lanthanons will form insoluble compounds with hydroxyl, phosphate, and bicarbonate ions The behavior of thorium, lanthanum, and uranium towards a number of physiologically important molecules was reported by Neuberg & Grauer (1953) According to this investigation, some of the most significant phosphorylated intermediates of carbohydrate metabolism were precipitated It is interesting to note that Kawin (1953) reported that when lanthanum is excreted in the urine, part of the element exists in an ionized form while the rest is bound to a complex with amino acids

Steidle (1935) reported that lanthanons precipitate proteins *in vitro* Rosoff et al (1958) found that yttrium *in vitro* is bound to bovine albumin and that the binding is directly proportional to the metal's concentration The complex formed with yttrium is, however, reversible and is greatly influenced by temperature and pH In rat serum, yttrium, cerium, promethium, and ytterbium

Table V Most important data about the radioisotopes used (International Atomic Energy Agency 1959)

Isotope	Chemical Formula	Half Life	Radiation Energy in MeV beta	Radiation Energy in MeV gamma	Specific Activity	Total Solids	Supplier
Y^{90}	YCl_3	58 days	0 36 (0 2 %) 1 55 (∼100 %)	1 19 (0 2 %)	Carrier free	1 mg /mC	Radiochemical Centre Amersham, England
Ce^{144}	$CeCl_3$	285 days	0 18 (24 %) 0 26 (≲5 %) 0 31 (76 %)	0 081 (3 %) 0 100 (1 5 %) 0 134 (8 5 %)	Carrier free	1 mg /mC	”
via Pr^{144}		17 5 min	0 80 (2 %) 2 3 (1 %) 3 0 (97 %)	0 69 (∼2 %) 1 5 (∼0 3 %) 2 2 (∼0 8 %)			
Pm^{147}	$PmCl_3$	2 6 years	0 22	—	Carrier-free	2 mg /mC	”
Tb^{160}	$TbCl_3$	73 days	0 37 (14 %) 0 56 (51 %) 0 86 (35 %) 1 71 (0 3 %)	0 087[1] 0 30[1] 0 88[1] 0 97[1] 1 18[1] 1 27[1]	0 05 mC/mg Tb	No data available	”
Ho^{166}	$HoCl_3$	27 3 hours	0 23 (2 %) 0 41 (5 %) 0 87 (9 %) 1 76 (37 %) 1 84 (47 %)	0 08 (∼6 %) 1 38 (∼1 %) 1 61 (weak) 1 69 (weak)	0 5 mC/mg Ho	”	”
Yb^{169}	$YbCl_3$	31 days	—	0 008 to 0 31 (12 gammas)	0 1 mC/mg Yb	”	”

[1] All of low abundance.

CHAPTER II

Material and Methods

Experimental Animals

In all of the experiments, male and female white rats of the Sprague Dawley race were used They were about two months old and weighed 150—225 grams The rats were fed with a standard pelleted food

Isotopes

The following rare earth metals have been investigated yttrium, cerium, promethium, terbium, holmium, and ytterbium Radioisotopes and stable isotopes have both been used All of these were injected as chlorides

Physical data of importance for the investigation of the radioisotopes are given in Table V Immediately before the radioelements were injected the pH was adjusted with sodium hydroxide to 3 0—3 5 The doses of the radioisotopes are given in Table VI

According to the description of the supplier, the stable isotopes had a purity of 99 5 per cent or higher Stable elements were injected as aqueous solution with a pH between 5 and 6

Narcosis of the Experimental Animals

The animals were anesthetized with Mebumal® intraperitoneally, the dose being 24 mg per kilogram body weight Intravenous injections, excretory investigations, and decapitation with collection of blood was always done on completely anesthetized animals

Administration of the Isotopes

Injections were performed into a femoral vein which had been dissected free A one ml syringe graded in 1/100 ml was used When standards were prepared (chapter III), the syringe and needle were weighed before and after injection The volume injected varied between 0 10 and 0 20 ml

Bile Collection

Bile was collected through a polyethylene catheter (PE 10, 0 28/0 60 mm) from the common bile duct which was incised about one cm above Vater s

TABLE V Most important data about the radioisotopes used (International Atomic Energy Agency 1959)

Isotope	Chemical Formula	Half Life	Radiation Energy in MeV beta	gamma	Specific Activity	Total Solids	Supplier
Y^{91}	YCl_3	58 days	0.36 (0.2 %) 1.55 (∼100 %)	1.19 (0.2 %)	Carrier free	1 mg./mC	Radiochemical Centre Amersham England
Ce^{144}	$CeCl_3$	285 days	0.18 (24 %) 0.26 (≤5 %) 0.31 (76 %)	0.081 (3 %) 0.100 (1.5 %) 0.134 (8.5 %)	Carrier free	1 mg./mC	"
via Pr^{144}		17.5 min	0.80 (2 %) 2.3 (1 %) 3.0 (97 %)	0.69 (∼2 %) 1.5 (∼0.3 %) 2.2 (∼0.8 %)			
Pm^{147}	$PmCl_3$	2.6 years	0.22	—	Carrier free	2 mg./mC	"
Tb^{160}	$TbCl_3$	73 days	0.37 (14 %) 0.56 (51 %) 0.86 (35 %) 1.71 (0.3 %)	0.087[1] 0.30[1] 0.88[1] 0.97[1] 1.18[1] 1.27[1]	0.05 mC/mg Tb	No data available	"
Ho^{166}	$HoCl_3$	27.3 hours	0.23 (2 %) 0.41 (5 %) 0.87 (9 %) 1.76 (37 %) 1.84 (47 %)	0.08 (∼6 %) 1.38 (∼1 %) 1.61 (weak) 1.69 (weak)	0.5 mC/mg Ho	"	"
Yb^{169}	$YbCl_3$	31 days	—	0.008 to 0.31 (12 gammas)	0.1 mC/mg Yb	"	"

[1] All of low abundance

TABLE VI Dosage expressed as μC per rat of radioactive isotopes

	Chapter III	Chapter IV
Y^{91}	80[1]	20
	10[2]	
Ce^{144}	80[1]	5
	10[2]	
Pm^{147}	80[1]	5
	10[2]	
Tb^{160}	80[1]	5
	10[2]	
Ho^{166}	80[1]	10[3]
	10[2]	25[4]
Yb^{169}	80[1]	5
	10[2]	

[1] In experiments with 1—3 hours' duration
[2] ,, ,, ,, 1—4 days' duration
[3] ,, ,, ,, 0 03—24 hours' duration
[4] ,, ,, ,, 48—192 hours' duration

papilla When bile flowed freely, the abdominal incision was closed Collection started immediately after the administration of a radioisotope The collection periods for bile were 20 minutes

Collection of Urine

Urine was taken from female rats through a polyethylene catheter (PE 50, 0 58/0 95 mm) which was inserted into the bladder through the urethra

Differential Centrifugation of Cell Fractions

After the animals had been decapitated and exsanguinated, the liver was immediately removed and cooled in running tap water One gram of liver was homogenized in 20 ml of 0 25 M sucrose (pH 5) with the aid of a MSE homogenizer Smears were made so as to ensure that the liver cells were crushed After homogenization, which took 4 minutes, the homogenates were put into an ice bath

Ten ml of the homogenate were centrifuged in a Spinco Ultracentrifuge Model L, with rotor 40 Differential centrifugation was performed largely according to Hogeboom & Schneider (1955) Four fractions were obtained *Fraction 1* consisted mainly of nuclei spun down at 1000 g for 15 minutes *Fraction 2*—"mithochondrial fraction"—was obtained after a further 15 minutes but at 10,000 g, *Fraction 3*—"microsome fraction"—was obtained after a 60 minute centrifugation at 100,000 g The supernatant after fraction 3 consisted of the cytoplasmic matrix, *Fraction 4* Smears of the fractions were made

and checked in a light microscope This was done on unstained smears as well as smears stained with haemalum eosin and Giemsa After each centrifugation, one ml of the supernatant was pipetted off for a determination of radioactivity This volume was replaced by one ml of the original liver homogenate

The following was seen in the smears The nuclear fraction contained nuclei and also a few cell fragments consisting mainly of mitochondria This was not unexpected as heavy mitochondria generally will be found in a nuclear fraction The smears of the mitochondrial fraction were very uniform, dominated by granules which by all criteria appeared to be mitochondria The microsome fraction appeared homogeneous and lacked the granules in fraction 2

From a cytological point of view cell fractions obtained through differential centrifugation were not completely homogeneous This was particularly the case with the nuclear fraction The intermixing was, however, slight and each fraction was strongly dominated by its characteristic component

Distribution of Radioactivity in Cell Fractions

Radionuclide distribution in the various cell fractions was determined by measuring the activity of the original liver homogenate and the activity of each of the supernatants of fractions 1, 2, and 3 The differences in radioactivity correspond to the uptake in particles spun down The radioactivity was expressed as per cent of the total amount in the original liver homogenate and calculated as follows

A = radioactivity in liver homogenate (cpm /ml)
B = radioactivity in A—nuclear fraction (cpm /ml)
C = radioactivity in B—mitochondrial fraction (cpm /ml)
D = radioactivity in C—microsome fraction (cpm /ml)

The uptake of radioactivity in the nuclear fraction =

$$= A \text{ (cpm)} - B \text{ (cpm)} = \frac{(A-B)\ 100}{A} \%$$

The uptake of radioactivity in the mitochondrial fraction =

$$= B \text{ (cpm)} - C \text{ (cpm)} = \frac{(B-C)\ 100}{A} \%$$

The uptake of radioactivity in the microsome fraction =

$$= C \text{ (cpm)} - D \text{ (cpm)} = \frac{(C-D)\ 100}{A} \%$$

The uptake of radioactivity in the supernatant (fraction 4) =

$$= D \text{ (cpm)} = \frac{100\ D}{A} \%$$

Preparation of Organs for Determination of Radioactivity

From the liver 0 5—1 0 gram of tissue was taken Only one kidney was used for the kidney analysis The gastro intestinal tract was dissected free from the mesentery and divided into the following sections 1) the stomach 2) the duodenum (the oral 12 cm of the small intestine), 3) the oral half of the jejunum 4) the aboral half of the jejunum, including the ileum 5) the caecum and 6) the colon The stomach was rinsed with 10 ml of saline while the various intestinal segments were rinsed with 10—20 ml of the same solution The rinsing solution was collected as quantitatively as possible

All organs were wet ashed as described by Ekman (1961) Serum bile urine, and cell fractions were measured directly but brought to a constant volume by the addition of water

Preparation of Radioactive Standards

When dilutions were prepared for injection from stock radioisotope solutions a standard was also prepared To the standard solution hydrochloric acid was added so as to give a final pH of less than 2 Standards for GM measurements were made through wet ashing organs from an untreated rat Standards for bile serum and urine were also prepared by the addition of a known volume of the radioisotope solution Although samples and standards were measured on a fixed volume of one ml the same amount of matter was always present and correction for self absorption could be omitted (Comar 1955) For the radioactive determinations in connection with the differential centrifugation a correction for self absorption was done since every sample contained a different amount of matter

Determination of Radioactivity

As is seen in Table V all radionuclides studied with the exception of Pm emit γ rays Pm is a pure β emitter Determination of γ activity was done with a scintillation well crystal whereas β activity was determined with GM tubes The efficiencies of the determinations were as follows

Y	3 5 per cent
Ce	1 8
Pm	0 1
Tb	6 3
Ho	1 5
Yb	6 8

Radioactivity in the livers was generally high and gave a counting error (Comar 1955) of less than one per cent When determinations were done on the

kidneys the counting error was between 1 and 3 per cent The activities in blood serum samples and samples of gastro-intestinal contents were sometimes so low that the counting error reached a value of ± 10 per cent

Determination of Blood Glucose

This was done according to the method of Lunden (1963) Blood (0 05 ml) was taken from the jugular vein and pipetted directly into perchloric acid From 15 double determinations the error of the method was calculated to be ± 4 7 per cent at normal glucose values

Determination of Ornithine Carbamyl Transferase (OCT)

These determinations were performed according to the method of Reichard (1957) The error of the method as calculated from 15 double determinations was found to be ± 10 9 per cent at normal values

Histologic Investigation

A 10 per cent formalin solution Bouin s fluid and absolute ethyl alcohol were used as fixatives Routinely the paraffin sections were stained *with haemalum* eosin In some cases the sections were also stained with van Gieson s method PAS iron staining according to Hueck, and fibrin staining according to Weigert In order to demonstrate fat staining with Scharlach Rot was used Glycogen was demonstrated by staining the material fixed in absolute ethyl alcohol with B st s carmine stain

Electron Microscopic Investigation

Immediately after sacrifice small pieces of liver tissue *were fixed for two* hours in ? per cent osmium tetroxide solution buffered to pH 7 3—7 5 in a temperature of +4° C The material was embedded in Epon Epoxy Resin (Luft 1961) The sections were prepared with an Ultratome (LKB) picked up on uncoated copper grids stained with uranyl acetate and investigated with a Siemens Elmascope I

Statistical Analyses

Statistical analyses were performed by Miss Kerstin Hallgard at Dep[illegible] of [illegible]

we [illegible] formulae were used

Preparation of Organs for Determination of Radioactivity

From the liver 0.5—1.0 gram of tissue was taken. Only one kidney was used for the kidney analysis. The gastro-intestinal tract was dissected free from the mesentery and divided into the following sections: 1) the stomach, 2) the duodenum (the oral 12 cm. of the small intestine), 3) the oral half of the jejunum, 4) the aboral half of the jejunum, including the ileum, 5) the caecum and 6) the colon. The stomach was rinsed with 10 ml. of saline while the various intestinal segments were rinsed with 10—20 ml. of the same solution. The rinsing solution was collected as quantitatively as possible.

All organs were wet ashed as described by Ekman (1961). Serum, bile, urine, and cell fractions were measured directly but brought to a constant volume by the addition of water.

Preparation of Radioactive Standards

When dilutions were prepared for injection from stock radioisotope solutions, a standard was also prepared. To the standard solution, hydrochloric acid was added so as to give a final pH of less than 2. Standards for GM measurements were made through wet ashing organs from an untreated rat. Standards for bile, serum, and urine were also prepared by the addition of a known volume of the radioisotope solution. Although samples and standards were measured on a fixed volume of one ml., the same amount of matter was always present and correction for self-absorption could be omitted (Comar 1955). For the radioactive determinations in connection with the differential centrifugation, a correction for self-absorption was done, since every sample contained a different amount of matter.

Determination of Radioactivity

As is seen in Table V, all radionuclides studied with the exception of Pm^{147} emit γ-rays. Pm^{147} is a pure β emitter. Determination of γ activity was done with a scintillation well crystal whereas β activity was determined with GM-tubes. The efficiencies of the determinations were as follows:

Y^{91}	3.5 per cent
Ce^{144}	1.8 „ „
Pm^{147}	0.1 „ „
Tb^{160}	6.3 „ „
Ho^{166}	1.5 „ „
Yb^{169}	6.8 „

Radioactivity in the livers was generally high and gave a counting error (Comar 1955) of less than one per cent. When determinations were done on the

CHAPTER III

Excretion of Y^{91}, Ce^{144}, Pm^{14}, Tb^{160}, Ho^{166}, and Yb^{169}

The liver and skeleton occupy a key position in the metabolism of the rare earth metals On the basis of the great affinity which the lanthanons have for liver tissue, it is reasonable to assume that their excretion into the intestine takes place by way of the bile This, their probable bile excretion, has been a subject for discussion (Hamilton 1947, Durbin et al 1956) From the available literature, the bile excretion of the lanthanons seems to be inadequately studied It has been shown that, in the rat, cerium is excreted by way of the bile (Castellino 1961, Castellino et al 1962) Knowledge of the bile excretion of the remainder of the lanthanons seems to be completely lacking In order to obtain a better understanding of the excretion of the lanthanons as a whole, the present work was carried out The objective of the experiment was to study the following factors

1 The concentration of lanthanons in blood serum,
2 The uptake of lanthanons by the kidneys,
3 The excretion of lanthanons via urine,
4 The uptake of lanthanons by the liver,
5 The excretion of lanthanons via bile,
6 The excretion of lanthanons by way of the stomach and intestinal tract

In the experiments, attention was primarily directed toward the initial excretion during the first three hours after an intravenous injection This depended, among other things, upon the technical execution of the experiments, which was associated with many difficulties (narcosis operation etc) The rats—only females were used—were killed after one, two, and three hours The con centration of lanthanons in the serum and liver was also determined one, two, and four days after administration In connection with this, the bile excretion was studied during the hour before the experimental animals were killed For each time period, four rats, as a rule, were killed A total of 140 rats was used in the experiments

Results

1 *The concentration in blood serum*

The blood serum concentration of the lanthanons being investigated dropped with varying speeds (Figs 2 a—b) during the first hours after the intravenous

x single observation
n number of observation
ε error of method
d difference between two determinations
df degrees of freedom

m mean of x, $m = \frac{\Sigma x}{n}$

s standard deviation, $s = \sqrt{\frac{\Sigma (x - m)^2}{n - 1}}$

t test, $t = \frac{m_1 - m_2 -}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$ with $df = n_1 - n_2 - 2$

ε error of method $= \sqrt{\frac{\Sigma d^2}{2n}}$ with $df = n$

ce counting error of the radioactive measurements (=standard deviation of sample counting rate [Comar 1955])

$$ce = \pm \sqrt{\frac{C_s}{T_s^2} + \frac{C_b}{T_b^2}},$$

where C_s = total counts for sample in time T_s
C_b = total counts for background in time T_b

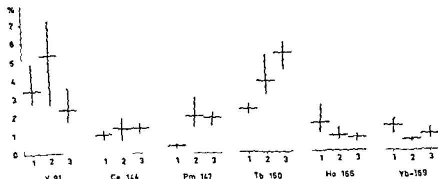


Figure 3 The concentration in the kidneys of Y^{91}, Ce^{144}, Pm^{147}, Tb^{160}, Ho^{166}, and Yb^{169} expressed as per cent of the given dose 1, 2, and 3 hours after intravenous administration As a rule the diagram shows the mean and range values from four rats

injection After two hours, the concentrations of holmium-166 and ytterbium-169 were about five per cent of the given dose per ml blood serum, while corresponding values for the remaining nuclides investigated were less than one per cent The drop in concentration was most pronounced for yttrium-91 The serum contained, thus only 0 30 per cent of the given dose per ml one hour after administration Holmium-166 and ytterbium-169 were strikingly different from the remaining lanthanons These two metals dropped appreciably slower in the blood than did the others This was particularly the case with ytterbium 169 which, one hour after injection, existed in a concentration of about ten per cent of the given dose per ml of blood serum During one to four days after administration, the content of lanthanons in the blood dropped even further After two days, all of the nuclides investigated had, in general, a concentration lower than 0 01 per cent of the given dose per ml blood serum All of the isotopes had, however, a measurable concentration after four days with the exception of holmium-166 The fact that holmium-166 could not be demonstrated at this time was probably due to its short half life (27 3 hours) The serum concentration after two and four days was almost the same for yttrium 91, cerium 144, promethium-147, and ytterbium-169

2 *The uptake by the kidneys*

The concentration of the lanthanons investigated in the kidneys generally reached a few per cent of the given dose (Fig 3) Yttrium 91 and terbium-160 with 2—5 per cent of the given dose appeared in the highest amounts in the kidneys In most of the experiments, the kidneys contained 1—2 per cent of

Figure 2 a The concentration of Y^{91}, Pm^{147}, and Tb^{160} per ml blood serum after intravenous administration As a rule, each value is the mean of four single values

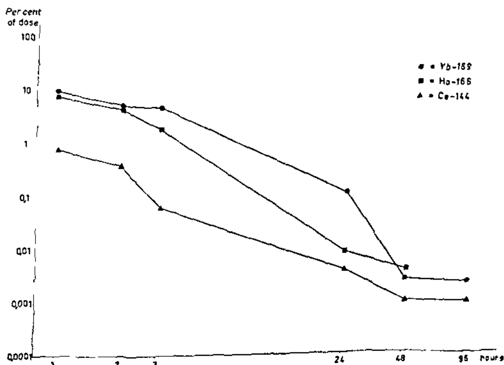


Figure 2 b The concentration of Ce^{144}, Ho^{166}, and Yb^{169} per ml blood serum after intra venous administration As a rule, each value is the mean of four single values

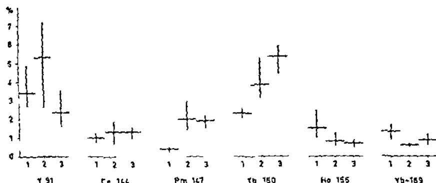


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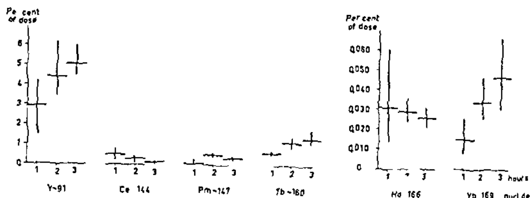


Figure 4 The excretion of Y^{91}, Ce^{144}, Pm^{147}, Tb^{160}, Ho^{166}, and Yb^{169} in the urine within *1*, *2*, and *3* hours after intravenous administration As a rule, the diagram shows the mean and range values from four rats

the given dose of cerium-144 and promethium-147 One hour after the injection, the kidneys held more than one per cent and two hours after, less than one per cent of the given dose of holmium-166 and ytterbium-169

3 *The excretion via urine*

All of the lanthanons investigated were excreted via the urine (Fig 4) Yttrium-91 showed the largest total excretion level with about 5 per cent of the given dose during the first 2—3 hours after injection Less than two per cent of the given dose of terbium-160 was excreted in the first three hours Corresponding values for cerium-144 and promethium-147 were, as a rule, lower than 0 50 per cent of the given dose Holmium-166 and ytterbium-169 had the lowest concentrations in the urine of all the nuclides The excretion of these two elements in the first three hours was generally less than 0 050 per cent of the given dose

4 *The uptake by the liver*

In all of the investigations done, the liver showed a high content of the investigated lanthanons (Fig 5) Yttrium-91 occupied, to a certain degree a unique position as its uptake by the liver was lower than that of the other elements investigated The maximum content of yttrium-91 in the liver was 25—30 per cent of the given dose This value was obtained after both two and three hours Some days after the intravenous injection, the liver contained about 10 per cent of the given dose of Y^{91} The content of Ce^{144} and Pm^{147} in the liver was about the same at each of the successive examination times Their uptake by the liver was, in every case, more than 50 per cent of the given dose The

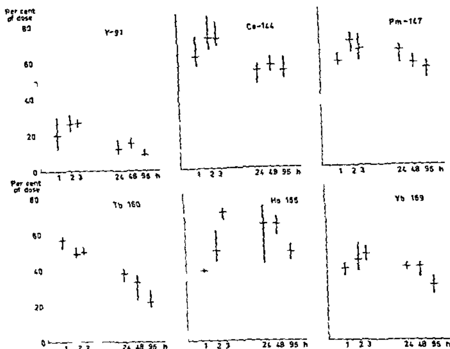


Figure 5 The uptake in the liver of Y^{91}, Ce^{144}, Pm^{147}, Tb^{160}, Ho^{166}, and Yb^{169} at different times after intravenous administration As a rule the diagram shows the mean and range values from four rats

maximal amount of these two lanthanons in the liver was about 70 per cent of the given dose This was obtained a few hours after administration Ce^{144} and Pm^{147} showed the largest and quickest uptake in the liver Their liver concentrations fell more slowly than those of the remaining lanthanons The maximal uptake of Tb^{160} by the liver was obtained as early as one hour after the injection and was 55 per cent of the given dose In the subsequent investigational periods, the liver concentration of Tb^{160} gradually fell Four days after administration, the liver contained about 25 per cent of the given dose Ho^{166} had a somewhat slower uptake by the liver than had the remaining nuclides investigated At some examination times (after 3, 24, and 48 hours), the liver contained amounts of Ho^{166} as high as 60—70 per cent of the given dose Next to Y^{91} Yb^{169} showed the lowest liver uptake of the lanthanons investigated The highest amount (45—50 per cent of the given dose) was measured three hours after administration Four days after this, the liver concentration of Yb-169 was about 30 per cent of the given dose

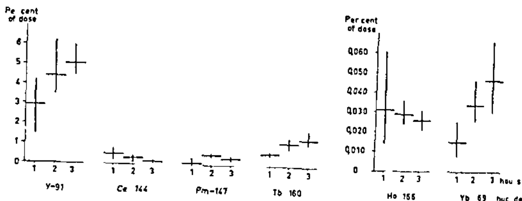


Figure 4 The excretion of Y^{91}, Ce^{144}, Pm^{147}, Tb^{160}, Ho^{166}, and Yb^{169} in the urine within 1, 2, and 3 hours after intravenous administration As a rule, the diagram shows the mean and range values from four rats

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All of the lanthanons investigated were excreted via the urine (Fig 4) Yttrium-91 showed the largest total excretion level with about 5 per cent of the given dose during the first 2—3 hours after injection Less than two per cent of the given dose of terbium-160 was excreted in the first three hours Corresponding values for cerium-144 and promethium 147 were, as a rule, lower than 0 50 per cent of the given dose Holmium 166 and ytterbium 169 had the lowest concentrations in the urine of all the nuclides The excretion of these two elements in the first three hours was generally less than 0 050 per cent of the given dose

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In all of the investigations done, the liver showed a high content of the investigated lanthanons (Fig 5) Yttrium 91 occupied, to a certain degree a unique position as its uptake by the liver was lower than that of the other elements investigated The maximum content of yttrium 91 in the liver was 25—30 per cent of the given dose This value was obtained after both two and three hours Some days after the intravenous injection, the liver contained about 10 per cent of the given dose of Y^{91} The content of Ce^{144} and Pm^{147} in the liver was about the same at each of the successive examination times Their uptake by the liver was, in every case, more than 50 per cent of the given dose The

Figure 6 b The bile excretion of Ce^{144} under the same experimental conditions as in Figure 6 a

Figure 6 c. The bile excretion of Pm^{147} under the same experimental conditions as in Figure 6 a.

5 *The excretion via bile*

All of the lanthanons investigated showed an *excretion via bile* although in varying degrees (Figs 6 a—f) *Holmium-166 and ytterbium 169* with 0 53 per cent and 0 45 per *cent, respectively*, *of* the given dose accumulated the largest excretion *during the first three* hours after intravenous injection Cerium-144 had the lowest bile excretion (0 081 per cent of the given dose) for the same time During the same period of time, the total excretion of *yttrium-91, promethium*-147, and terbium-160 was 0 20 per cent, 0 19 per cent, and 0 30 *per cent of* the given dose, respectively *Following* the intravenous injection, the *bile excretion* of *the investigated* nuclides quickly reached a maximum For *holmium*-166 and ytterbium-169, *this was* reached *after* approximately two *hours and for* the remaining isotopes, after 20—40 minutes When maximal *bile excretion* had *been* reached, the different lanthanons dropped with vary*ing* speeds The most rapid were yttrium-91, cerium-144, and *promethium 147 After 2—3* hours, the excretion of these, taken on a twenty *minute sample*, was *less* than 0 01 *per cent of* the given dose As is stated above, holmium 166 and ytterbium-169 had the largest bile excretion and thus remained at a com

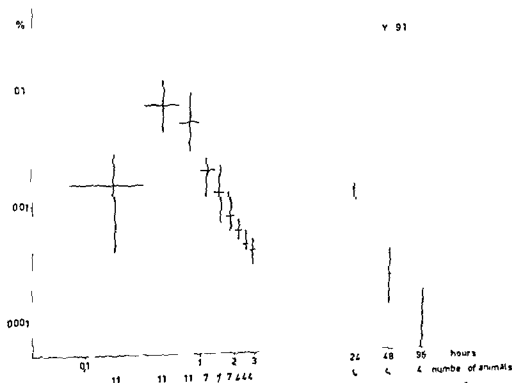


Figure 6 a The mean and range values for the excretion of Y⁹¹ via bile expressed as per cent of the given dose Each value represents the excretion during a 20 minute period

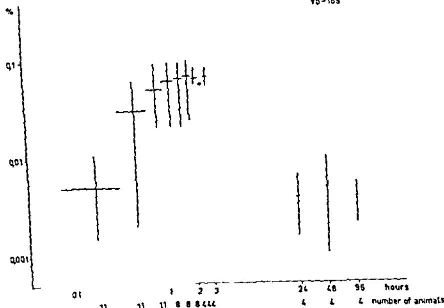


Figure 6 f The bile excretion of Yb^{169} under the same experimental conditions as in Figure 6 a

paratively high level for the first hours after administration Three hours after the injection, the two latter named metals were excreted via bile at the rate of 0 060 per cent of the given dose in a twenty minute sample One, two, and four days after intravenous administration, the excretion via bile was appreciably lower than it was initially during the first three hours In general, the excretion of lanthanons was lower after one day than after two and four days Cerium 144 showed the lowest excretion (0 004 per cent of the given dose during the 24th hour) and holmium-166 the largest (0 035 per cent of the given dose during the 48th hour) when the bile excretion was studied *one to* four days after administration

6 *The excretion by way of the stomach and intestinal tract*

The lanthanons investigated could always be demonstrated in the contents of the gastro-intestinal tract (Figs 7 a—g) The total content of the various nuclides in the stomach and intestinal tract was in about the same proportion (0 10—0 60 per cent of the given dose) during the initial excretion The greatest activity was often found in the stomach contents In many cases, the activity decreased with samples taken in an aboral direction

3 Magnusson

%

Tb-160

0,1

0,01

0,001

0,1 1 2 3 24 48 96 hours

11 11 11 7 7 7 4 4 4 4 4 4 number of animals

Figure 6 d The bile excretion of Tb^{160} under the same experimental conditions as in *Figure 6* a

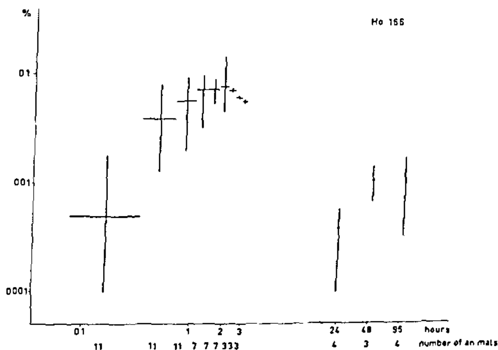
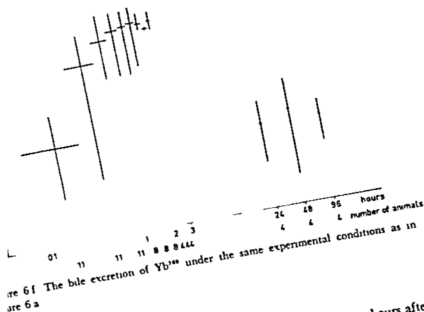


Figure 6 e The bile excretion of Ho^{166} under the same experimental conditions as in Figure 6 a



ure 6 f The bile excretion of Yb^{169} under the same experimental conditions as in
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Figure 7 a The excretion of Y^{91} via the gastro-intestinal tract expressed as per cent of the given dose 1, 2 and 3 hours after intravenous administration Each value represents the excretion in a) stomach, b) duodenum c) oral jejunum d) aboral jejunum, e) caecum and f) colon resp As a rule the diagram shows the mean and range values from four rats

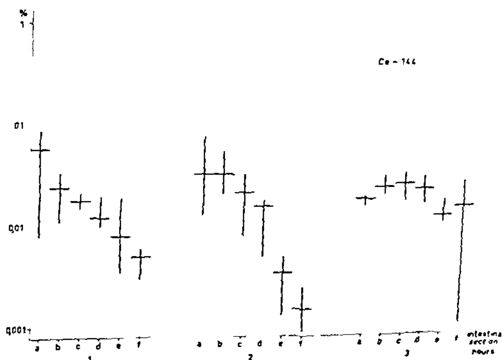


Figure 7 b The excretion of Ce^{144} via the gastro-intestinal tract under the same experimental conditions as in Figure 7 a

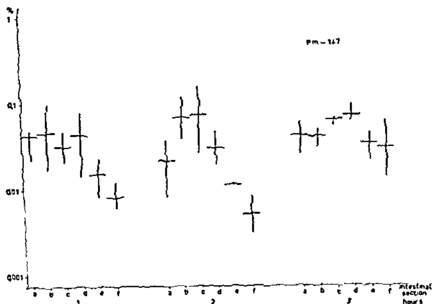


Figure 7 c The excretion of Pm[147] via the gastro-intestinal tract under the same experimental conditions as in *Figure 7 a*.

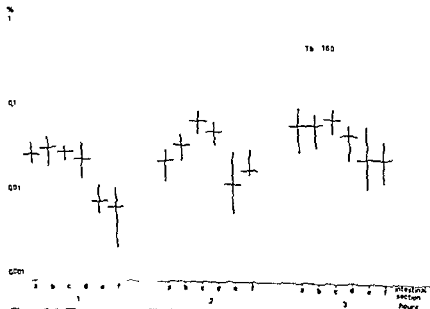


Figure 7 d The excretion of Tb[160] via the gastro-intestinal tract under the same experimental conditions as in Figure 7 a.

%
1

Y-91

0.1

0.01

0.001

a b c d e f
1

a b c d e f
2

a b c d e f
3

intestinal section
hours

Figure 7a The excretion of Y^{91} via the gastro-intestinal tract expressed as per cent of the given dose 1, 2, and 3 hours after intravenous administration. Each value represents the excretion in a) stomach, b) duodenum, c) oral jejunum, d) aboral jejunum, e) caecum, and f) colon, resp. As a rule, the diagram shows the mean and range values from four rats.

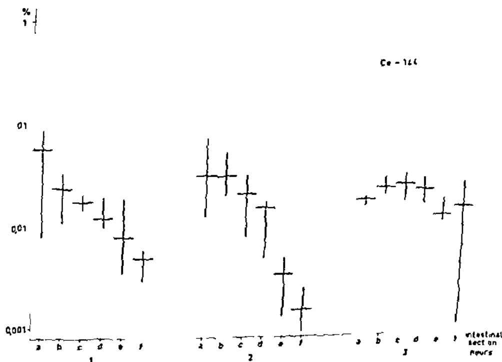


Figure 7b The excretion of Ce^{144} via the gastro-intestinal tract under the same experimental conditions as in Figure 7a.

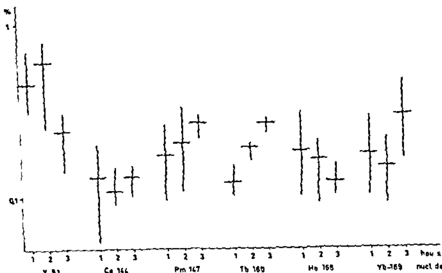


Figure 7 g The total excretion of Y^{91}, Ce^{144}, Pm^{147}, Tb^{160}, Ho^{166}, and Yb^{169} via the gastro-intestinal tract expressed as per cent of the given dose 1, 2 and 3 hours after intravenous administration As a rule the diagram shows the mean and range values from four rats

Discussion

In tracer solutions radioactive isotopes occur in a low chemical concentration These have under certain conditions a strong tendency to form radiocolloids (Schweitzer & Jackson 1952 Schweitzer 1956) One factor which influences the formation of radiocolloids is the pH of the solution Investigations of the physical chemical states of radiocerium (Aeberhardt 1961) showed that $Ce^{III}Cl_3$ was completely ionized in a pH lower than 4 5 In all probability, a similar behavior prevails for the solutions of the remaining lanthanon salts, so that there is a pH value under which the salt is completely ionized In order to avoid the formation of radiocolloids as much as possible, the pH in the injection solutions was held at 3 0—3 5 Under these conditions, the lanthanons used in the experiments for the intravenous injections ought to have been highly ionized

The speed with which the lanthanons leave the blood depends, among other things upon their physical-chemical states The blood concentration of radiocerium in colloidal form drops much more rapidly than for the same isotope in ionized form (Aeberhardt et al 1962) The serum concentration of lanthanons found during the investigations agrees, to a large extent, with the results from earlier work Schubert et al (1950) report about the same blood con-

Figure 7 e The excretion of Ho^{166} via the gastro intestinal tract under the same experimental conditions as in Figure 7 a

Figure 7 f The excretion of Yb^{169} via the gastro intestinal tract under the same experimental conditions as in Figure 7 a

1961) The fact that light lanthanons have a greater uptake by the liver is also in accordance with earlier experiences However, holmium-166 which has a remarkably high uptake in the liver does not always follow this rule Cerium-144 and promethium-147, of all the lanthanons investigated, attain the most rapid and the largest uptake by the liver The amounts of these two metals also maintain a higher level (somewhat more than 50 per cent of the given dose) for a longer period of time than the others This behavior can possibly be interpreted as an expression that they (Ce^{144} and Pm^{147}) are strongly bound to the parenchyme of the liver The investigation shows that a strong fall in blood concentration follows a rapid rise in the maximal uptake level in the liver (see cerium 144, yttrium 91, promethium-147, and terbium 160) On the other hand, a slower drop in blood concentration is connected with a slower uptake in the liver This is best demonstrated by holmium-166 Of the lanthanons investigated, yttrium-91 appeared in the lowest amount in the liver This is to be somewhat expected however, as yttrium is a pronounced bone seeker On the other hand, it is significantly taken up by the liver as seen in the present investigation. The relatively high initial uptake of yttrium should therefore, from a radiobiological point of view, be observed more than thus far has been the case A similar interpretation has also been put forth by Spode (1958) The same author also says that yttrium is transported from the liver to the skeleton to be deposited there The fact that a similar transport mechanism should also apply to cerium 144 is made applicable by Aeberhardt et al (1962)

The concentration of nuclides in the kidneys and urine shows, in the short time during which the investigation was being done, some differences between the various lanthanons Yttrium-91 and terbium 160 have a constantly high content in the kidneys but they seem to behave somewhat differently Yttrium-91 reaches its maximal value (5—6 per cent of the given dose) two hours after administration while that of terbium 160 occurs in a successively rising uptake The amount in the kidneys gives a fairly good picture of the concentration in the urine Thus yttrium-91 and terbium-160 give the highest activities in the urine The lower amount of yttrium-91 in three hours than in the two earlier investigations can be closely compared to the higher concentration in the urine In contrast to this, the concentrations of terbium-160 in the kidneys and in the urine rise side by side Clearly, a direct concentration of terbium-160 in the kidneys must occur Cerium-144 and promethium-147 are found in lower concentrations in the kidneys than yttrium 91 and terbium-160 and also have a lower urine excretion The lowest concentrations in the kidneys and urine come from holmium 166 and ytterbium-169 The amount of holmium 166 in the kidneys drops and ytterbium-169 has the same tendency here also This can be dependent upon a fall in the blood concentration, an increased urine excretion or a combination of these two factors With hol-

centration for yttrium-91 The rapid fall of the amount of yttrium in the blood has been emphasized by Kyker et al (1954) Durbin et al (1956) states a plasma concentration for cerium-144 and terbium-160 of the same amount as in the present investigation In guinea pigs, the blood concentration falls somewhat more slowly than in rats with yttrium-91 (Spode 1958) as well as cerium-144 (Spode & Gensicke 1961) Aeberhardt et al (1962) found approximately the same blood concentration for ionized radiocerium as has been shown in the present experiments

Radioactive measurements in the present investigation were done on serum instead of whole blood The lanthanons should only be partially incorporated into the corpuscular elements Thus, cerium-144 is not taken up by the erythrocytes and thrombocytes but is incorporated into the nucleic acids of the leukocytes (Aeberhardt 1961) The concentration of lanthanons in the serum should, therefore, be in close agreement with that found in whole blood

The results found show that heavy lanthanons—represented here by Ho^{166} and Yb^{169}—fall *significantly more slowly in the blood than do the light ones* The experiment done by Durbin et al (1956) followed the same lines This dissimilarity in the blood concentration of lanthanons has particularly been *pointed out by Durbin (1962)*

The graphic illustration of the serum concentration of lanthanons (Figs 2 a—b) shows a predominant, continuous fall up to two days after the intravenous injection Then the concentration drops more slowly

Following intravenous administration, chemical reactions certainly occurred in the blood so that the ions of the lanthanons were bound in various ways *Through knowledge of the biochemical reactions of the rare earth metals* there are many alternatives which merit thought Schubert et al (1950) mention four important possibilities 1) combination with anions such as phosphate so that compounds which are difficult to dissolve are formed, 2) binding with the proteins, 3) polymerization of the hydroxide molecules of the lanthanons, and 4) formation of soluble complexes such as, citrate ions Yttrium-90 is transported bound to serum phosphatides (Graul & Hundeshagen 1959) Serum albumins play an important role in forming complexes with Y^{91}, Ce^{144}, Pm^{147}, and Yb^{169} (Ekman et al 1961) Presumably other possibilities for the transport of lanthanons in the blood also exist It is certain that chemical reactions which begin with a lanthanon in ionized form behave differently in the organism after administration than those administered in colloidal form (Aeberhardt et al 1962)

All of the investigated lanthanons were strongly concentrated in the liver as early as one hour after intravenous administration This confirms earlier investigations done on rats (Durbin et al 1956, Aeberhardt et al 1961 1962) as well as mice (Laszlo et al 1952, Lewin et al 1954 b Rosoff et al 1963) and guinea pigs (Spode 1958 Graul & Hundeshagen 1959 Spode & Gensicke

1961) The fact that light lanthanons have a greater uptake by the liver is also in accordance with earlier experiences However, holmium 166 which has a remarkably high uptake in the liver does not always follow this rule Cerium 144 and promethium 147, of all the lanthanons investigated, attain the most rapid and the largest uptake by the liver The amounts of these two metals also maintain a higher level (somewhat more than 50 per cent of the given dose) for a longer period of time than the others This behavior can possibly be interpreted as an expression that they (Ce^{144} and Pm^{147}) are strongly bound to the parenchyme of the liver The investigation shows that a strong fall in blood concentration follows a rapid rise in the maximal uptake level in the liver (see cerium 144, yttrium 91, promethium 147, and terbium 160) On the other hand a slower drop in blood concentration is connected with a slower uptake in the liver This is best demonstrated by holmium 166 Of the lanthanons investigated, yttrium 91 appeared in the lowest amount in the liver This is to be somewhat expected, however, as yttrium is a pronounced bone-seeker On the other hand, it is significantly taken up by the liver as seen in the present investigation The relatively high initial uptake of yttrium should therefore from a radiobiological point of view, be observed more than thus far has been the case A similar interpretation has also been put forth by Spode (1958) The same author also says that yttrium is transported from the liver to the skeleton to be deposited there The fact that a similar transport mechanism should also apply to cerium 144 is made applicable by Aeberhardt et al (1962)

The concentration of nuclides in the kidneys and urine shows in the *short* time during which the investigation was being done, some differences between the various lanthanons Yttrium 91 and terbium 160 have a constantly high content in the kidneys but they seem to behave somewhat differently Yttrium 91 reaches its maximal value (5—6 per cent of the given dose) two hours *after* administration while that of terbium 160 occurs in a successively rising uptake The amount in the kidneys gives a *fairly good picture of the* concentration in the urine Thus, yttrium 91 and *terbium 160* give the highest activities in the urine The lower amount of yttrium 91 *in three hours* than in the two earlier investigations can be closely compared to the higher concentration in the urine In contrast to this, the concentrations of terbium 160 [illegible]

[illegible] *in the* kidneys than yttrium 91 and terbium 160 and also have a lower urine excretion The lowest concentrations *in* the kidneys and urine come from holmium 166 and *ytterbium* 169 The amount of *holmium* 166 in the kidneys drops and *ytterbium 169* has the same tendency here also This can be dependent upon a fall *in* the blood concentration an increased urine excretion or a combination of these two factors With hol

mium-166, the fall in blood concentration should be reflected in the kidneys. With Yb^{169} there is, however, a question whether a successively increased urine excretion is followed by a lower content in the kidneys. The low excretion of *holmium-166 and ytterbium 169* via urine is remarkable when one considers that the heavy lanthanons are chiefly excreted via the kidneys (Durbin et al. 1956). It is an obvious fact that urine excretion has been studied during a very borderline time period and that it is *impossible to judge with any degree* of certainty what happens during the hours immediately following. Durbin et al. (*1955*) followed the excretion of lanthanons via urine for 256 days. According to these investigations, the urine excretion of Ce^{144} during the first day was 5.8 per cent of the given dose and of Yb^{169}, 17.4 per cent of the given dose. *The present experiments should, nevertheless, have given a certain understanding* of the initial urine excretion. The concentration of cerium-144 is in agreement with earlier investigations in rats (Aeberhardt et al. 1962). In guinea pigs, the kidneys contain a higher content of yttrium 91 (Spode 1958) as well as cerium-144 (Spode & Gensicke 1961). Yttrium 91 is excreted through tubular *excretion as well* as by glomerular filtration (Kawin *1955*).

According to earlier experiments, the rare earth metals are excreted, among other routes, via the gastro-intestinal tract (Durbin et al. 1956, Durbin 1960, 1962). For this route of excretion, there are many possibilities worth consideration. The way which is situated closest to hand and is most direct is via the bile with consideration being given to the rare earth metals' great affinity for liver tissue. Pancreatic juices are also a possible excretion route. This alternative is very unlikely since the lanthanons, with intravenous administration *are only insignificantly concentrated by the pancreas (Spode 1958,* Spode & Gensicke 1961, Ewaldsson & Magnusson 1963 a, 1963 b). The higher uptake which is obtained with intraperitoneal injection should, in all probability, be dependent upon a surface resorption (Spode 1958, Graul & Hundeshagen 1959, Spode & Gensicke 1961). A third possibility is an excretion directly through *the gastro-intestinal tract. The fact that this can occur is known.* This applies to foreign molecules such as dextran (Engstrand & Åberg 1950, Åberg 1952, Åberg et al. 1961) and to normal molecules such as serum albumin (Ullberg et al. 1960). Since the rare earth metals are transported in the blood while bound to albumins (Ekman et al. 1961), a gastro intestinal excretion should not be unreasonable. *The investigation which has been done* furnishes proof that such an excretion really does take place. A confirmation of this fact has also been obtained by the use of the autoradiographic method *(Ewaldsson* & Magnusson 1963 a, 1963 b). Even if the experiments clearly show a qualitative gastro intestinal excretion, it is very hazardous or even impossible to judge with any degree of certainty *the quantities of the various* lanthanons. In addition, the experimental conditions are subject to a rather large source of error. Likewise, there exist rather large biological variations in *reference to the secre*

tions of the gastric and intestinal juices However, the experiments intimate that the excretion is greatest in the stomach and decreases in the intestine, aborally This appears best in the experiments with a one hour duration The form in which the metals are excreted is an open question which requires further investigations There are various possibilities worth consideration They may occur as free ions, bound to serum albumin, and/or bound to other proteins

The suggestion of eventual bile excretion of the rare earth metals is completely justified This is shown by the results in the present experiment One can judge from the results that some differences in the excretion of light and heavy lanthanons occur The former have an appreciably lower bile excretion than the latter which are seen most of all during the first three hours after injection As stated above, these possibilities can depend upon the fact that the light lanthanons are strongly bound to the liver cells The relatively large excretion of holmium 166 and ytterbium 169 is possibly connected with their slow fall in concentration in the blood The fact that there is a rather large bile excretion of all of the lanthanons investigated during times when the blood concentration is moderately high—disregarding the first twenty minutes—may indicate that a certain time is required in order to insure a stronger binding to the liver cells Yttrium 91, which in many other respects is reminiscent of the heavy lanthanons has with reference to bile excretion, a greater similarity to the light lanthanons After a high initial bile excretion, it seems to decrease to a minimum and then rises again For yttrium 91, this minimum occurs at the earliest two to three hours after injection For the remaining lanthanons it occurs after one day This has also been observed by Castellino *et al* (*1962*) who measured a maximal bile excretion of cerium 144 four to five days after injection In the investigation just cited, a significantly larger bile excretion was shown than was found in the present investigation Perhaps the explanation lies in the different method which has come into use The form in which the lanthanons are excreted with bile can also be thought to vary Cerium 144 when excreted in bile, was bound to proteins (*Castellino* 1961 Castellino et al 1962) Other possibilities cannot be completely ruled out for example their binding to bile acids or their occurrence as free ions

In conclusion attention is merited by Durbin et al (1955) who have published complete analyses of the urinary and fecal excretion of lanthanons in rats These investigations stretched from 1—256 days As an example, the fecal excretion of Ce^{144} can be mentioned which, at day *one*, *was 1 0 per cent of the* given dose After five days, the accumulated excretion via feces was 6 0 per cent of the given dose and after 64 days, 59 2 per cent of the given dose As is seen these results support the significance of gastro-intestinal *excretion*

CHAPTER IV

The Subcellular Distribution of Y^{91}, Ce^{144}, Pm^{147}, Tb^{160}, Ho^{166}, and Yb^{169} in Liver Cells

The liver plays an important role in the metabolism of the rare earth metals After intravenous administration, the lanthanons are *rapidly concentrated in* the liver During the first days after the injection, the liver contains more than 50 per cent of the given dose If certain lanthanons (from lanthanum through to samarium) are given intravenously in a *toxic dose to rats, sex differences* can be observed In female rats, these elements produce fatty livers, however this is not the case in the male rats (Kyker et al 1957 a, Snyder et al 1959) It is possible that these differences between the sexes and the various lantha nons occur in a certain relation to the subcellular distribution of the metals within the liver cells According to the literature, the subcellular distribution of the lanthanons in liver cells has been little studied It is considered to be of value, therefore, to investigate with the help of differential centrifugation the distribution of lanthanons in the organelles of the liver cells The aims of the investigation were

1 To determine the amount of Ce^{144} and Yb^{169} in various cell fractions 24 hours after intravenous administration,
2 To obtain a qualitative determination of the various *cell fractions contents* of investigated lanthanons at various times after intravenous administration

The rats were killed after 2, 5, and 40 minutes and 2, 12, 24, 48, 96 and 192 hours, respectively At each time, two male and two female rats were investigated In reference to Ce^{144} and Yb^{169}, an additional twelve male and female rats were investigated 24 hours after administration A total of 264 rats was used

Results

1 *The subcellular distribution of Ce^{144} and Yb^{169} 24 hours after administration*

The investigation of the subcellular distribution of Ce^{144} (Table VII) and Yb^{169} (Table VIII) in liver cells 24 hours after intravenous administration shows that the nuclear fraction had the lowest concentration of both nuclides It is further clear that the nuclear and mitochondrial fractions contents of

Ce^{144} and Yb^{169} were significantly higher in male rats than in female rats In reference to the microsome fraction, the behavior was reversed Of the various cell fractions, the microsome fraction had the highest uptake of Ce^{144} as well as Yb^{169} in female rats The male rats had, however, the highest concentration of Ce^{144} in the supernatant and of Yb^{169} in the mitochondrial fraction and the supernatant.

A statistical comparison between the amounts of Ce^{144} and Yb^{169} in the different liver cell fractions 24 hours after intravenous administration is shown in Tables IX and X. The nuclear fraction had, at this time, the lowest amount of Ce^{144} as well as Yb^{169} in both of the sexes Comparison between the amounts in the various cell fractions shows that for both of the investigated nuclides there existed a significant difference between the nuclear fraction and the remaining three cell fractions in both male and female rats It can be seen from the same comparison that a highly significant difference existed between the contents of the mitochondrial and supernatant fractions only in reference to Ce^{144} in female rats Between the mitochondrial and microsome fractions, a highly significant difference in the contents of nuclides existed in reference to Ce^{144} in female rats and Yb^{169} in male rats. Between the contents in the microsome fraction and the supernatant, there existed a highly significant difference only with reference to Yb^{169} and male rats.

A statistical comparison (Table XI) between the amounts of Ce^{144} and Yb^{169} shows that a highly significant difference between both nuclides existed in the mitochondrial fraction in the female rats The uptake of Yb^{169} was greatest in this fraction In the male rats the same comparison shows a significant difference between the contents of both nuclides in the nuclear, mitochondrial, and microsome fractions Ce^{144} had its greatest uptake in the nuclear and microsome fractions while that of Yb^{169} occurred in the mitochondrial fraction

2 *The subcellular distribution at various times after administration*

The distribution of the investigated nuclides in the liver cell fractions at various times after intravenous administration is illustrated in Figures 8 a—f The motive for the investigation was to confirm whether or not the rare earth metals appear in the various liver cell fractions

From the results obtained it can be seen that male and female rats showed a liver cell uptake of all of the investigated nuclides It can also be seen that such was the case with all of the time periods from 2 minutes up to and including 192 hours after intravenous administration

TABLE VII Subcellular liver distribution of Ce^{144} in female (n = 12) and male (n = 12) rats 24 hours after intravenous administration

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Value	5 7	13 0	19 6	28 7	38 0	27 8	36 7	30 5
Standard Deviation	3 2	4 5	3 2	2 4	7 4	5 9	5 9	4 8
	P<0 1 %***		P<0 1 %***		0 1 %<P<1 %**		1 %<P<5 %*	

TABLE VIII Subcellular liver distribution of Yb^{169} in female (n = 12) and male (n=12) rats 24 hours after intravenous administration

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Value	6 3	9 0	29 0	34 8	34 0	21 6	31 3	34 6
Standard Deviation	1 9	1 6	4 5	5 2	4 9	3 7	3 7	5 9
	0 1 %<P<1 %**		0 1 %<P<1 %**		P<0 1 %***		10 %<P<25 %-	

Table IX A statistical comparison between the content of Ce^{144} in the various liver cell fractions in female and male rats based on values in Table VII

	Female			Male		
	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction
Supernatant	$P<0.1$ %***	$P<0.1$ %***	$P>25$ %-	$P<0.1$ %***	$P>25$ %-	$10\ \% < P < 25$ %-
Microsome Fraction	$P<0.1$ %***	$P<0.1$ %***		$P<0.1$ %***	$P>25$ %-	
Mitochondrial Fraction	$P<0.1$ %***			$P<0.1$ %***		

TABLE VII Subcellular liver distribution of Ce '' in female (n = 12) and male (n = 12) rats 24 hours after intravenous administration

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Value	5.7	13.0	19.6	28.7	38.0	27.8	36.7	30.5
Standard Deviation	3.2	4.5	3.2	2.4	7.4	5.9	5.9	4.8
	P<0.1 %***		P<0.1 %***		0.1 %<P<1 %**		1 %<P<5 %*	

TABLE VIII Subcellular liver distribution of Yb '' in female (n = 12) and male (n=12) rats 24 hours after intravenous administration

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Value	6.3	9.0	29.0	34.8	34.0	21.6	31.3	34.6
Standard Deviation	1.9	1.6	4.5	5.2	4.9	3.7	3.7	5.9
	0.1 %<P<1 %**		0.1 %<P<1 %**		P<0.1 %***		10 %<P<25 %-	

TABLE IX. A statistical comparison between the content of Ce^{144} in the various liver cell fractions in female and male rats based on values in TABLE VII

	Female			Male		
	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction
Supernatant	P<0.1 %***	P<0.1 %***	P>25 %	P<0.1 %***	P>25 % -	10 %<P<25 % -
Microsome Fraction	P<0.1 %***	P<0.1 %***		P<0.1 %***	P>25 % -	
Mitochondrial Fraction	P<0.1 %***			P<0.1 %***		

Table VII. Subcellular liver distribution of Ce^{144} in female (n = 12) and male (n = 12) rats 24 hours after intravenous administration.

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Value	5.7	13.0	19.6	28.7	38.0	27.8	36.7	30.5
Standard Deviation	3.2	4.5	3.2	2.4	7.4	5.9	5.9	4.8
	P<0.1 %***		P<0.1 %***		0.1 %<P<1 %**		1 %<P<5 %*	

Table VIII. Subcellular liver distribution of Yb^{169} in female (n = 12) and male (n=12) rats 24 hours after intravenous administration.

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Female	Male	Female	Male	Female	Male	Female	Male
Mean Value	6.3	9.0	29.0	34.8	34.0	21.6	31.3	34.6
Standard Deviation	1.9	1.6	4.5	5.2	4.9	3.7	3.7	5.9
	0.1 %<P<1 %**		0.1 %<P<1 %**		P<0.1 %***		10 %<P<25 %–	

TABLE IX A statistical comparison between the content of Ce^{++} in the various liver cell fractions in female and male rats based on values in TABLE VII

	Female			Male		
	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction
Supernatant	P<0.1 %***	P<0.1 %***	P>25 %	P<0.1 %***	P>25 % -	10 %<P<25 % -
Microsome Fraction	P<0.1 %***	P<0.1 %***		P<0.1 %***	P>25 % -	
Mitochondrial Fraction	P<0.1 %***			P<0.1 %***		

TABLE X A statistical comparison between the content of Yb^{169} in the various liver cell fractions in female and male rats based on values in TABLE VIII

	Female			Male		
	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction
Supernatant	P<0.1 %***	10 %<P<25 % -	10 %<P<25 % -	P<0.1 %***	P>25 % -	P<0.1 %***
Microsome Fraction	P<0.1 %***	1 %<P<5 %*		P<0.1 %***	P<0.1 %***	
Mitochondrial Fraction	P<0.1 %***			P<0.1 %***		

TABLE XI Comparison between the subcellular distribution of Ce^{144} and Yb^{169} in rat livers 24 hours after intravenous administration

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Ce^{144}	Yb^{169}	Ce^{144}	Yb^{169}	Ce^{144}	Yb^{169}	Ce^{144}	Yb^{169}
Female	5 7	6 3	19 6	29 0	38 0	34 0	36 7	31 3
	P>25 %–		P<0 1 %***		10 %<P<25 %–		1 %<P<5 %*	
Male	13 0	9 0	28 7	34 8	27 8	21 6	30 5	34 6
	0 1 %<P<1 %**		0 1 %<P<1 %**		0 1 %<P<1 %**		5 %<P<10 %–	

TABLE X A statistical comparison between the content of Yb^{169} in the various liver cell fractions in female and male rats based on values in TABLE VIII

	Female			Male		
	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction
Supernatant	P<0.1 %***	10 %<P<25 %-	10 %<P<25 %-	P<0.1 %***	P>25 %-	P<0.1 %***
Microsome Fraction	P<0.1 %***	1 %<P<5 %*		P<0.1 %***	P<0.1 %***	
Mitochondrial Fraction	P<0.1 %***			P<0.1 %***		

TABLE XI *Comparison between the subcellular distribution of* Ce^{+++} and Yb^{+++} in rat livers 24 hours after intravenous administration

	Nuclear Fraction		Mitochondrial Fraction		Microsome Fraction		Supernatant	
	Ce^{+++}	Yb^{+++}	Ce^{+++}	Yb^{+++}	Ce^{+++}	Yb^{+++}	Ce^{+++}	Yb^{+++}
Female	5 7	6 3	19 6	29 0	38 0	34 0	36 7	31 3
	P>25 %-		P<0 1 %***		10 %<P<25 %-		1 %<P<5 %*	
Male	13 0	9 0	28 7	34 8	27 8	21 6	30 5	34 6
	0 1 %<P<1 %**		0 1 %<P<1 %**		0 1 %<P<1 %**		5 %<P<10 %-	

Table X A statistical comparison between the content of Yb^{169} in the various liver cell fractions in female and male rats based on values in Table VIII

	Female			Male		
	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction	Nuclear Fraction	Mitochondrial Fraction	Microsome Fraction
Supernatant	P<0 1 %***	10 %<P<25 %-	10 %<P<25 %-	P<0 1 %***	P>25 %-	P<0 1 %***
Microsome Fraction	P<0 1 %***	1 %<P<5 %*		P<0 1 %***	P<0 1 %***	
Mitochondrial Fraction	P<0 1 %***			P<0 1 %***		

Figure 8 c The subcellular distribution of Pm^{147} in the rat liver under the same experimental conditions as in Figure 8 a

Tb 16

Figure 8 d The subcellular distribution of Tb^{160} in the rat liver under the same experimental conditions as in Figure 8 a.

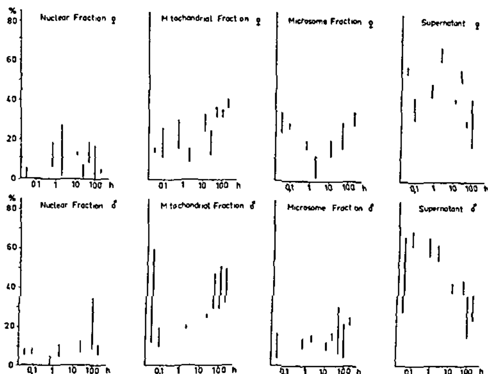


Figure 8 a The subcellular distribution of Y^{91} in the rat liver at different times after intravenous administration At each time, the line is drawn between the individual values of two rats

Figure 8 b The subcellular distribution of Ce^{144} in the rat liver under the same experimental conditions as in Figure 8 a

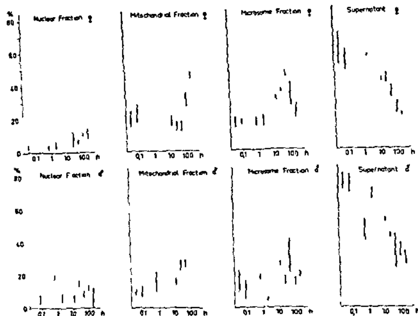


Figure 8 c The subcellular distribution of Pm^{147} in the rat liver under the same experimental conditions as in Figure 8 a

Tb 160

Nuclear Fraction ♀ Mitochondrial Fraction ♀ Microsome Fraction ♀ Supernatant ♀

Nuclear Fraction ♂ Mitochondrial Fraction ♂ Microsome Fraction ♂ Supernatant ♂

% 80 60 40 20 0

0.1 1 10 100 h

Figure 8 d The subcellular distribution of Tb^{160} in the rat liver under the same experimental conditions as in Figure 8 a.

Figure 8 c The subcellular distribution of Ho^{166} in the rat liver under the same experimental conditions as in Figure 8 a

Figure 8 f The subcellular distribution of Yb^{169} in the rat liver under the same experimental conditions as in Figure 8 a

Discussion

Only Catsch et al (1961) have investigated the subcellular distribution of a lanthanon ($Ce^{\cdots}$) However, the subcellular distribution of the rare earth metals might well add information as to why these metals show varying toxicities and produce various changes in the liver

The present investigation was performed primarily to give information about the behavior of the lanthanons at various times after administration Therefore, investigations at various time periods were carried out on a small number of animals The small number of animals used in each time period makes it impossible to draw significant quantitative conclusions. In spite of the material being rather borderline in this aspect, certain observations can be made All of the investigated lanthanons could as a rule be demonstrated in the various cell fractions each time a sample was taken The nuclear fractions, in most cases had the lowest amount of the investigated nuclides Possibly, a more substantial uptake of the heavy lanthanons in the nuclear fraction existed in male rats four and eight days after administration The mitochondrial fraction showed an uptake for all of the investigated lanthanons and for both sexes. This uptake following administration increased with time The microsome fraction probably had a more constant uptake of lanthanons In the supernatant as a rule the amounts of all the lanthanons in both sexes following administration sank with time The initial high amounts which existed in the supernatant and thereafter dropped show that the lanthanons are probably first deposited in the cytoplasmic matrix of the liver cell From this, then, transportation to the various cellular organelles might later occur

The investigation of Catsch et al (1961) gave in principle the same results on cerium 144 as the present one Only male rats were used by Catsch et al (1961) and the only time comparable with the present investigation was the 24 hour investigation The small differences are probably due to differences in separation techniques Their values for the nuclear fraction and the mitochondrial fraction are in complete agreement with those here presented Catsch et al (1961) point out that great changes in the distribution pattern in the liver cells exist during the first days after intravenous administration of radiocerium

Cerium is more toxic to female rats than to male rats The heavy lanthanons which were investigated are however, roughly equally toxic to both sexes The rare earth metals in toxic doses, produce liver damage The light lanthanons cause fatty changes which, with equal dosing to both sexes are demonstrable only in females The heavy lanthanons cause focal liver necroses which with the same dose to both sexes can be demonstrated in both female and male rats. In order to seek a possible connection between the toxic effects of the lanthanons on the liver and the subcellular distribution in the liver, one light ($Ce^{\cdots}$) and one heavy ($Yb^{\cdots}$) lanthanon were investigated on a larger

number of animals (Tables VII and VIII) which thus permitted statistical analyses. From the results, it is evident that Ce^{144} shows a significant sex difference in all cell fractions and, with the exception of the supernatant fraction, that this is also the case for Yb^{169}. A comparison between cerium-144 and ytterbium-160 shows, in female rats, a highly significant difference only between the amounts in the mitochondrial fraction. However, in male rats, there was a significant difference between the amounts in all cell fractions except the supernatant.

At present, no conclusions as to the significance of these differences and the varied toxicological behavior of these lanthanons in males and females can be drawn with any certainty. There is a possibility that the results of the subcellular distribution mirror biochemical patterns in the liver cell. It is tempting to presume that in the case of cerium, the subcellular distribution differences mirror the occurrence of fatty liver. Should this be the case, however, ytterbium would not behave like cerium if both had the same chemical influence on the chemical functions of the cell organelles. There is a great difference in the toxicities of these lanthanons on a weight basis. The mechanism by which they influence the intracellular metabolism in detail is, of course, still completely unknown.

CHAPTER V

Acute Toxic Effect of Yttrium, Cerium, Terbium, Holmium, and Ytterbium on the Liver

The prominent role of the liver in the metabolism of the rare earth metals has been pointed out earlier in this work With intravenous administration the lanthanons are concentrated rapidly and to a great extent in the liver If certain rare earth metals are injected intravenously into rats in toxic doses, morphological as well as functional changes in the liver can be demonstrated (Kyker et al 1957 a Snyder et al 1959 1960 a 1960 b Glenn et al 1962) It is primarily the fatty liver caused by lanthanons which has been studied from different aspects The toxicity of the lanthanons in general is less well studied (Kyker & Cress 1957) An increased knowledge of the toxic effects of the rare earth metals is considered to be desirable The aims of this experiment were

1 To study the influence of the rare earth metals on the blood glucose level
2 To investigate the extent of acute liver damage by determination of ornithine-carbamyl transferase (OCT) in the blood serum
3 To confirm the acute liver damage morphologically by macroscopic, microscopic and electron microscopic investigations

After intravenous administration of the lanthanons the liver status was followed by investigations at different times (Table XII) When the rats were killed blood was collected for determination of glucose and OCT The blood sugar and OCT levels were determined over a ten day period Selected material was used for microscopic and electron microscopic investigations For the former primarily those livers which could be macroscopically seen to contain changes were chosen For the latter cases were selected where the OCT values showed liver damage A total of 458 rats was used

In order to induce acute liver damage a certain toxic dose had to be used Partly the occurrence of death and partly increased OCT values were chosen as measures of the toxic effect The mortality and the OCT values are given in Tables XII and XIV a—e respectively These criteria of toxicity were fulfilled by the following doses

Lanthanon	Dose expressed as mg metal per kilogram body weight
Yttrium	9
Cerium	3
Terbium	35
Holmium	40
Ytterbium	60

TABLE XII Number of rats investigated at different times after intravenous administration of yttrium, cerium, terbium, holmium, and ytterbium When not stated otherwise, the rats were sacrificed

Time following injection (in days)	Yttrium		Cerium		Terbium		Holmium		Ytterbium	
	females	males	females	males	females	males	females	males	females	males
0 1	5	5	5	5	2	2	2	2	5	5
0 5	5	5	5	5	5	5c)	5c)	5c)	5	5
1	12a)	13d)	5	5	6c)	9f)	8f)	9g)	6c)	5
2	9b)	4c)	20e)	5	4	3	4	4	5	5
3	6c)	4	14e)	5	4	3	5	5	5	6c)
4	3	3	1	5	5	3	4	5	4	5
5	3	4	—	5	—	—	—	—	5	5
6	3	4	1	4	2	5	4	3	5	4
7	3	4	—	4	—	—	—	—	4	5
8	3	5	—	5	—	—	—	—	4	5
10	2	2	—	5	5	5	3	3	5	5

a) = 11 died spontaneously
b) = 2 " "
c) = 1 " "
d) = 12 " "
e) = 13 " "
f) = 4 " "
g) = 5 " "

Following intravenous administration of cerium to male rats in the doses given above, increased OCT values were obtained but no deaths were encountered The dose of cerium used produced only lesser acute toxic effects in male rats

Results

1 *Blood glucose changes*

The glucose concentration in the blood is given in Tables XIII a—e and Figures 9 a—b According to the results, the amount of glucose decreased soon after administration After 12 and 24 hours, the blood glucose values generally lay between 50 and 100 mg /100 ml in both sexes Two and three days after administration, the glucose values varied between 80 and 105 mg /100 ml in female and male rats This was true for all of the investigated lanthanons with the exception of cerium when injected into females For this metal, the glucose content of the blood in female rats at these times was less than 40 mg /100 ml As a rule, the glucose levels were within the normal limits of variation four days after administration

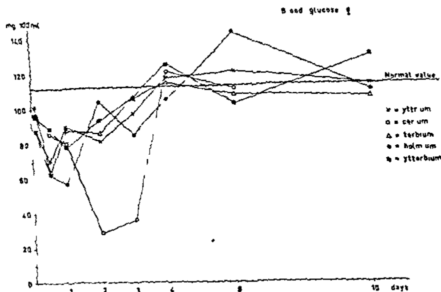


Figure 9a The concentration of blood glucose in female rats after intravenous administration of lanthanons based on values in Tables XIII a—e

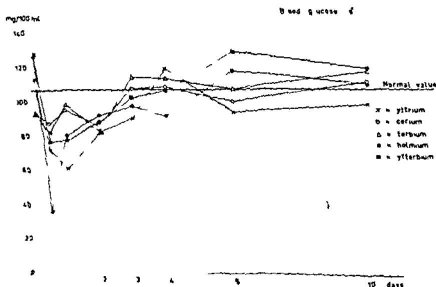


Figure 9b The concentration of blood glucose in male rats after intravenous administration of lanthanons based on values in Tables XIII a—e

TABLE XII Number of rats investigated at different times after intravenous administration of yttrium, cerium, terbium, holmium, and ytterbium When not stated otherwise, the rats were sacrificed

Time following injection (in days)	Yttrium		Cerium		Terbium		Holmium		Ytterbium	
	females	males	females	males	females	males	females	males	females	males
0 1	5	5	5	5	2	2	2	2	5	5
0 5	5	5	5	5	5	5[c]	5[c]	5[c]	5	5
1	12[a]	13[d]	5	5	6[c]	9[f]	8[f]	9[g]	6[c]	5
2	9[b]	4[c]	20[e]	5	4	3	4	4	5	5
3	6[c]	4	14[e]	5	4	3	5	5	5	6[c]
4	3	3	1	5	5	3	4	5	4	5
5	3	4	—	5	—	—	—	—	5	5
6	3	4	1	4	2	5	4	3	5	4
7	3	4	—	4	—	—	—	—	4	5
8	3	5	—	5	—	—	—	—	4	5
10	2	2	—	5	5	5	3	3	5	5

a) = 11 died spontaneously
b) = 2 " ,
c) = 1 " "
d) = 12 " ,
e) = 13 " "
f) = 4 , ,
g) = 5 " "

Following intravenous administration of cerium to male rats in the doses given above, increased OCT values were obtained but no deaths were encountered The dose of cerium used produced only lesser acute toxic effects in male rats

Results

1 *Blood glucose changes*

The glucose concentration in the blood is given in Tables XIII a—e and Figures 9 a—b According to the results, the amount of glucose decreased soon after administration After 12 and 24 hours, the blood glucose values generally lay between 50 and 100 mg/100 ml in both sexes Two and three days after administration, the glucose values varied between 80 and 105 mg/100 ml in female and male rats This was true for all of the investigated lanthanons with the exception of cerium when injected into females For this metal the glucose content of the blood in female rats at these times was less than 40 mg/100 ml As a rule, the glucose levels were within the normal limits of variation four days after administration

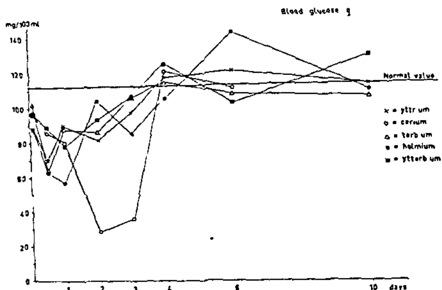


Figure 9 a. The concentration of blood glucose in female rats after intravenous administration of lanthanons based on values in Tables XIII a—e

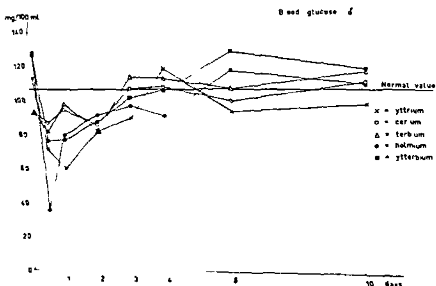


Figure 9 b. The concentration of blood glucose in male rats after intravenous administration of lanthanons based on values in Tables XIII a—e

TABLE XIII a The concentration of blood glucose (mg/100 ml) at different time intervals after intravenous administration of yttrium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	112	99—127	14	107	83—133
0 1	5	88	81—100	3	113	101—125
0 5	5	70	47—82	5	71	11—98
1	1	90	—	1	60	—
2	7	81	65—95	3	82	77—88
3	5	97	81—112	4	90	76—101
4	3	117	101—129	3	119	117—121
5	3	103	98—110	4	106	87—136
6	3	120	119—121	4	93	77—121
7	3	116	107—122	4	103	90—122
8	3	115	94—143	5	118	107—138
10	2	112	105—119	2	97	85—108

TABLE XIII b The concentration of blood glucose (mg/100 ml) at different time intervals after intravenous administration of cerium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	112	99—127	14	107	83—133
0 1	5	97	95—105	5	93	85—101
0 5	5	86	74—90	5	87	83—113
1	5	80	62—92	5	95	85—101
2	7	28	9—45	5	86	77—109
3	1	36	—	5	107	90—134
4	1	120	—	5	108	86—135
5	—	—	—	5	94	86—105
6	1	110	—	4	100	93—108
7	—	—	—	4	103	89—132
8	—	—	—	4	126	111—149
10	—	—	—	5	111	87—124

Table XIII c The concentration of blood glucose (mg/100 ml) at different time intervals after intravenous administration of terbium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	112	99—127	14	107	83—133
0.1	2	102	95—109	2	93	82—104
0.5	5	64	60—73	4	82	59—108
1	5	88	71—112	5	98	87—112
2	4	86	74—106	3	82	54—105
3	4	106	94—123	3	114	97—123
4	5	114	105—126	3	113	111—124
6	2	107	100—113	5	107	85—117
10	5	105	99—117	5	117	108—124

Table XIII d The concentration of blood glucose (mg/100 ml) at different time intervals after intravenous administration of holmium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	112	99—127	14	107	83—133
0.1	2	88	86—90	2	128	111—145
0.5	4	63	52—78	4	36	6—76
1	4	57	31—78	4	80	34—112
2	4	104	92—113	5	92	34—118
3	5	85	80—94	5	97	89—116
4	4	105	97—113	5	91	76—104
6	4	142	128—152	3	118	104—142
10	3	109	98—116	3	110	96—127

Table XIII e The concentration of blood glucose (mg/100 ml.) at different time intervals after intravenous administration of ytterbium

Time following injection in days	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	112	99—127	14	107	83—133
0.1	5	97	94—107	5	127	108—141
0.5	5	89	76—103	5	76	64—86
1	5	78	65—88	5	77	51—103
	5	93	84—101	5	87	75—94
3	5	106	94—121	5	102	62—116
4	4	125	110—139	5	106	93—130
5	5	110	97—117	5	113	90—126
6	5	102	91—129	4	128	103—141
7	4	114	101—127	5	103	86—127
8	4	106	99—120	5	119	111—127
10	5	128	119—137	5	118	95—143

2 Changes in OCT levels of blood serum

The OCT concentration in the blood serum is shown in Tables XIV a—e and Figures 10 a—b. During the first days after administration, an increase in the amount of OCT in the blood serum occurred with all of the rare earth metals investigated in both sexes. In some cases, such a rise was observed as early as two hours. The highest OCT values—often more than 100 Reichard units—were found 24 hours after administration except in those female rats injected with cerium. In the latter case, the concentration of OCT in blood serum reached the highest values—more than 500 Reichard units—after two to three days. From four days after the intravenous injection on the OCT levels found for all of the lanthanons investigated in both sexes could, as a rule, be placed within the normal limits.

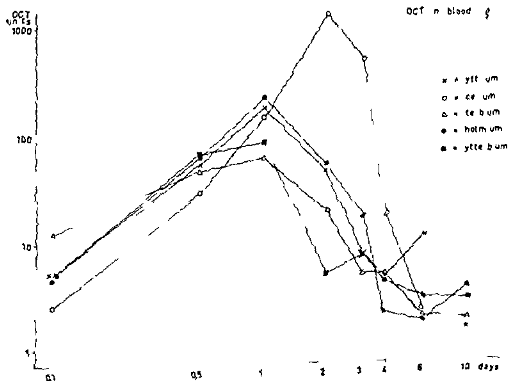


Figure 10 a. The blood serum concentration of ornithine—carbamyl—transferase (OCT) in females rats after intravenous administration of lanthanons based on values in Tables XIV a—e

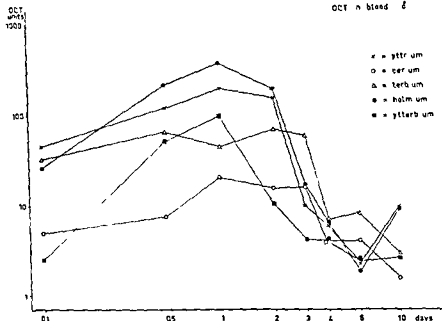


Figure 10 b The blood serum concentration of ornithine—carbamyl—transferase (OCT) in male rats after intravenous administration of lanthanons based on values in Tables XIV a—e

TABLE XIV a The concentration of ornithine-carbamyl transferase (Reichard units) in blood serum at different times after intravenous administration of yttrium.

Time following injection (in days)	Female rats Number of rats	Mean	Range	Male rats Number of rats	Mean	Range
Control	15	2.8	0.0—8.8	15	3.3	1.1—8.8
0.1	5	4.7	2.7—6.6	5	45.6	2.2—66.6
0.5	5	55.6	30.8—116.0	4	114.0	66.2—161.2
1	1	184.0	—	1	186.0	—
2	7	48.8	4.4—95.0	3	141.8	122.5—161.0
3	5	6.1	0.0—18.3	4	9.5	1.7—25.2
4	3	4.4	1.1—8.8	3	5.7	2.2—8.3
5	3	5.5	2.2—12.1	4	4.2	2.2—7.2
6	3	11.7	2.2—28.5	4	2.3	1.7—2.7
7	3	2.8	2.2—3.9	4	8.0	1.7—11.0
8	3	5.7	4.4—8.2	5	11.6	3.4—25.0
10	2	1.4	1.1—1.7	2	9.0	3.9—14.0

2 *Changes in OCT levels of blood serum*

The OCT concentration in the blood serum is shown in Tables *XIV* a—*c and* Figures *10* a—b During the first days *after administration*, an increase in the amount *of* OCT *in* the blood serum occurred *with all of the rare earth* metals investigated in both sexes In *some cases, such a rise* was observed as early as two hours The highest OCT values—*often more than 100* Reichard units—*were found 24 hours after administration except in those female rats injected* with cerium In the latter case, the concentration *of* OCT in blood serum *reached the highest* values—more than 500 Reichard units—after *two to three days* From four days after the intravenous *injection on, the OCT levels found for all of* the lanthanons investigated in both sexes could, as a rule, be placed *within the normal limits*

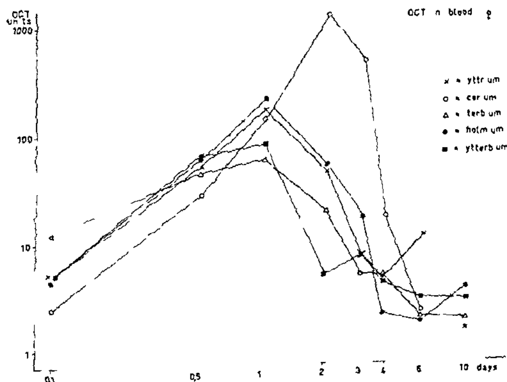


Figure 10 a The blood serum concentration of ornithine—carbamyl—transferase (OCT) in females rats after intravenous administration of lanthanons based on values in Tables XIV a—e

TABLE XIV d The concentration of ornithine-carbamyl transferase (Reichard units) in blood serum at different times after intravenous administration of holmium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	2 8	0 0—8 8	15	3 3	1 1—8 8
0 1	2	6 1	4 4—7 8	2	26 7	24 4—29 0
0 5	4	65 6	21 0—114 0	4	203 0	140 0—270
1	4	229 0	94 0—358 0	4	354 0	101 0—62(
2	4	55 5	5 0—177 0	5	180 6	43 0—58. .
3	5	17 2	6 8—42 0	5	15 9	2 0—49.5
4	4	2 1	1 1—3 3	5	6 4	2 2—14 4
6	4	1 7	0 0—4 4	3	1 8	1 1—2 7
10	3	3 7	2 2—4 4	3	8 8	3 4—19 0

TABLE XIV e The concentration of ornithine carbamyl transferase (Reichard units) in blood serum at different times after intravenous administration of ytterbium.

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	2 8	0 0—8 8	15	3 3	1 1—8 8
0 1	5	6 4	0 0—12 4	5	3 1	0 0—10 2
0 5	5	68 5	43 0—181 0	5	49 8	16 4—185
1	5	88 1	25 0—172 0	5	92 0	18 0—183
2	5	4 4	2 2—6 6	5	11 6	4 4—34
3	5	6 7	1 7—24 0	5	4 1	1 7—8 8
4	4	4 2	1 1—8 8	5	3 8	2 2—6
5	5	1 9	1 1—2 2	5	3 4	1 1—7
6	5	2 9	1 1—4 4	5	2 3	0 5—4
7	4	3 7	1 7—5 4	4	3 3	0 0—
8	4	5 8	0 0—14 4	5	7 4	2 2—
10	5	2 8	1 1—5 0	5	2.5	0 0—

Table XIV b The concentration of ornithine-carbamyl-transferase (Reichard units) in blood serum at different times after intravenous administration of cerium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	2 8	0 0—8 8	15	3 3	1 1—8 8
0 1	5	1 8	0 0—2 2	5	3 9	2 0—5 4
0 5	5	28 7	17 6—72 0	5	6 8	3 4—8 8
1	5	150 2	21 0—246 0	5	19 4	8 8—32 8
2	6	1390 0	960 0—1940 0	5	15 6	7 4—26 4
3	1	524 0	—	5	15 3	2 2—50 0
4	1	17 6	—	5	3 8	0 0—4 4
5	—	—	—	5	5 5	1 1—17 6
6	1	2 2	—	4	3 9	2 2—7 8
7	—	—	—	4	3 3	1 1—6 6
8	—	—	—	4	1 1	0 0—4 4
10	—	—	—	5	1 5	0 0—4 4

Table XIV c The concentration of ornithine carbamyl-transferase (Reichard units) in blood serum at different times after intravenous administration of terbium

Time following injection (in days)	Female rats			Male rats		
	Number of rats	Mean	Range	Number of rats	Mean	Range
Control	15	2 8	0 0—8 8	15	3 3	1 1—8 8
0 1	2	12 6	10 8—14 4	2	32 5	32 0—33 0
0 5	5	47 6	33 0—76 0	4	62 9	17 6—101 0
1	5	62 6	16 4—167 0	5	43 2	27 0—75 5
2	4	20 4	4 4—55 0	3	64 0	22 0—110 0
3	4	3 6	2 2—5 4	3	54 1	24 0—126 0
4	5	3 9	3 5—5 0	3	6 6	4 4—8 8
6	2	1 9	0 5—3 3	5	7 7	3 3—17 6
10	5	1 8	0 0—4 4	5	7 8	1 1 4 4

Cerium (females) In the liver, changes occurred in the form of a fatty degeneration This was observed in all liver lobuli, within the lobuli, the fatty degeneration had a diffuse spreading appearance A large number of fat droplets was often observed in the sections investigated (Fig 13) The fat droplets in the liver cells were of predominantly small sizes. Many of the liver cells showed necrobiotic nuclear changes in the form of karyopyknosis, karyorrhexis, and karyolysis Small intralobular necroses also occurred (Fig 14) These small areas of necrosis had no particular localization within the liver lobuli but could be seen in any part Fatty degeneration of the liver could be demonstrated one day after the intravenous administration of a toxic dose of cerium After this time, the changes were slight but increased during the third and fourth days so that they, in many cases, were of a particularly high degree Six days after the administration, no fatty changes of the liver were observed

The occurrence of glycogen in liver cells was investigated in 23 cases After twelve hours the presence of glycogen in the liver cells was considerably reduced After one day and during the second and third days after administration no glycogen was demonstrated in the liver cells. From the fourth day after the injection on, the liver cells again contained glycogen

Cerium (males) Signs of degeneration occurred in the liver cells Hydropic changes could be seen in the cytoplasm so that the liver cells, to a large extent, showed an empty appearance In a small number of cases, isolated necrotic liver cells and necrobiotic nuclear changes were also observed In two cases, there was a small number of liver cells which contained fat droplets The described changes mainly occurred from one to four days after the intravenous administration of a toxic dose of cerium

The occurrence of glycogen in the liver cells was investigated in 42 cases From twelve hours up to and including three days after the administration, a reduction in the amount of glycogen in the liver cells occurred This reduction of glycogen occurred chiefly in the central part of the liver lobuli where, in many cases practically no glycogen at all could be demonstrated From the fourth day on the liver cells exhibited no definite lowering of the glycogen level

Yttrium terbium holmium ytterbium (males and females) Histological investigation confirmed that the changes seen macroscopically were necroses Figs 15 a 15 b 16) These were intralobular and were, above all, localized to the midzonal region of the liver lobuli The necroses were well limited and had a round or if they were larger, a more irregular shape Sometimes the necroses extended around a large part of the midzonal region. The necroses did not exist in all of the lobuli but were limited to some of them. Occasionally the necroses showed hemorrhages. The first necroses were observed 12 hours after the administration of a toxic dose of these rare earth metals At this time,

3 *Morphological changes*

a) Macroscopic investigation

Cerium (females) The liver, as a rule, was of a normal size It had a very yellow color and a soft consistency The cut surface of the liver was also yellow and quite greasy This fatty liver (Fig 11) could be observed one day after the intravenous administration and was most pronounced on the third and fourth days after administration

Cerium (males) The liver showed no macroscopically observable changes with the exception of a slight enlargement

Yttrium, terbium, holmium, ytterbium (males and females) The liver showed a slight enlargement Often, focal necroses were observed in the liver (Fig 12) These appeared on the outer surface as well as on cut surfaces as rather distinctly demarcated but sometimes confluencing areas with diameters which in general, varied from a half to two mm The color of these necrotic areas varied The earliest observed foci (as a rule after two days) had a red yellow to yellow color Four to five days after the administration, they had a greyish tone These varying colors could also occur at the same time on the same liver so that the outer and the cut surfaces had a mottled appearance The number of foci in one and the same liver varied within rather wide limits Sometimes the liver contained only a few isolated areas of necrosis while another time it showed an abundance of them The foci could be seen in any part of the liver However, a certain concentration of the necrotic areas in the caudal lobe was observed This localization was most pronounced when the foci were numerous

b) Microscopic investigation

A survey of the material which became the subject of histological investigation is shown in Table XV From this table, the number of cases with a fatty degeneration and focal necroses is also evident

TABLE XV Survey of the microscropic investigation

Lanthanon	Number of livers histologically *investigated*		Number of livers with fatty degeneration		Number of livers with focal necroses	
	females	males	females	males	females	males
Yttrium	18	22	0	0	10	14
Cerium	53	32	41	0	0	0
Terbium	10	11	0	0	2	7
Holmium	11	12	0	0	8	10
Ytterbium	10	10	0	0	5	8

Cerium (females) Changes in the fine structure of the liver cells were demonstrated twelve hours after the administration of toxic doses of cerium Maximum changes were reached two to three days later The contrast of the cellular structures decreased The cytoplasmic matrix increased strongly in electron density and became compressed by swollen mitochondria, lipid droplets and vacuoles

The nuclei lost their normal round shape and became irregular in apperance In some cells, the nuclei were compressed by lipid droplets or vacuoles. The nuclei showed degenerative changes Thus the karyoplasm had a grainy appearance due to an irregular aggregation of chromatin A concentration of the chromatin in the periphery of the nucleus was also observed In some cell nuclei, osmiophilic inclusions of varying sizes were seen which were probably lipid droplets (Fig 18)

The mitochondria were enlarged Twelve hours after administration, their cross sections (smallest diameter) averaged 0 75 μ as opposed to 0 50 μ for the normal material The mitochondria often occupied the main part of the liver cells The electron density of the ground substance of the mitochondria decreased The mitochondrial cristae partly disappeared (Fig 19)

During the first day after administration, there was a dissociation of ribosomes from the endoplasmic reticulum in large areas of the liver cells The ribosomes were often disarranged in the cytoplasmic matrix (Fig 19) In some areas with a smooth endoplasmic reticulum, small, electron dense, clumps obviously consisting of aggregated ribosome were seen (Fig 20) The endoplasmic reticulum occasionally showed various degrees of dilatation so that the profiles assumed a rounded or irregular shape (Fig 21) The membranes were sometimes comparatively indistinct Two to three days after administration, liver cells were observed in which the endoplasmic reticulum without ribosomes but in the form of tightly packed, irregularly running membranes occupied a large portion of the cells (hypertrophy of the endoplasmic reticulum) Except for isolated lipid droplets no other components were seen in such areas of the liver cells (Fig 22)

One day after the intravenous administration of cerium, the liver cells contained cytoplasmic vacuoles (Fig 20) These often occurred in large numbers and were at least in some cases, surrounded by membranes The contents of the vacuoles showed a poor electron density at this time The contents later increased in density (Fig 21) and grew more osmiophilic (Fig 22) Comparison with light microscope observations suggested that the contents of the described vacuoles developed into lipid droplets At the same time, very small homogenous osmiophilic droplets (probably fat) were also observed which were surrounded by single membranes separated from the inclusion by a space (Fig 23)

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they were small. In livers investigated after a longer time interval the liver necroses were larger and involved large parts of the lobuli. In some cases the necroses spread to such a degree that they involved a major part of the lobuli. The largest necroses occurred two to three days after administration. From four days on the liver necroses showed cellular infiltration. These infiltrating cells consisted principally of histiocyte cells and a small number of neutrophile granulocytes. The histiocyte cells occurred mostly in the periphery of the necroses. In liver sections from rats sacrificed six to ten days after administration multinucleated giant cells were observed. In many cases the necrotic masses were surrounded by several *multinucleated giant cells* (Fig. 16). An occurrence of fat droplets within the liver cells was not, as a rule, demonstrated. In five cases out of 104, some of the liver cells adjoining the necroses contained small fat droplets. No hemosiderosis was demonstrated. With the fibrin staining no changes of the sinusoids were observed.

The investigation of glycogen amount in the liver cells was limited to those animals given yttrium. In reference to this metal 79 livers were investigated for glycogen. From twelve hours up to and including three days after the administration the liver cells showed a reduction in glycogen. In many cases the glycogen in the liver cells could not be demonstrated at all during this time. From the fourth day after injection on the amount of glycogen in the liver cells appeared to be normal.

c) Electron microscopic investigation[1]

The material for the electron microscopic investigation is summarized in Table XVI. This material was taken from two hours to four days after the intravenous administration of lanthanons. The ultrastructure was compared with that of a normal rat liver (Fig. 17). The observations on the fine structure of the normal livers were in accordance with the description given by Fawcett (1955).

TABLE XVI. Survey of the electron microscopic investigation

Lanthanon	Number of livers investigated females	males
Yttrium	6	2
Cerium	6	6
Terbium	1	1
Holmium	1	1
Ytterbium	1	1

[1] The electron microscopic work was performed in collaboration with Dr. Bo Crabo at the laboratory for electron microscopy (Head: Docent Nils Björkman), Royal Veterinary College, Stockholm. The investigation as reported here describes the main changes in the liver cells under the present experimental conditions. A detailed report will be published elsewhere.

Cerium (females) Changes in the fine structure of the liver cells were demonstrated twelve hours after the administration of toxic doses of cerium Maximum changes were reached two to three days later The contrast of the cellular structures decreased The cytoplasmic matrix increased strongly in electron density and became compressed by swollen mitochondria, lipid droplets, and vacuoles

The nuclei lost their normal round shape and became irregular in apperance In some cells, the nuclei were compressed by lipid droplets or vacuoles The nuclei showed degenerative changes. Thus the karyoplasm had a grainy appearance due to an irregular aggregation of chromatin A concentration of the chromatin in the periphery of the nucleus was also observed In some cell nuclei, osmiophilic inclusions of varying sizes were seen which were probably lipid droplets (Fig 18)

The mitochondria were enlarged Twelve hours after administration, their cross sections (smallest diameter) averaged 0 75 μ as opposed to 0 50 μ for the normal material The mitochondria often occupied the main part of the liver cells The electron density of the ground substance of the mitochondria decreased The mitochondrial cristae partly disappeared (Fig 19)

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One day after the intravenous administration of cerium, the liver cells contained cytoplasmic vacuoles (Fig 20) These often occurred in large numbers and were at least in some cases, surrounded by membranes The contents of the vacuoles showed a poor electron density at this time The contents later increased in density (Fig 21) and grew more osmiophilic (Fig 22) Comparison with light microscope observations suggested that the contents of the described vacuoles developed into lipid droplets. At the same time, very small homogenous osmiophilic droplets (probably fat) were also observed which were surrounded by single membranes separated from the inclusion by a space (Fig 23)

Cerium (males) The liver cells showed only slight changes Isolated liver cell nuclei had somewhat irregular shapes and on occasion showed concentration of the chromatin in the periphery of the nucleus The only observable change in the mitochondria was a slight enlargement In many liver cells the endoplasmic reticulum lost its ribosomes and exhibited dilatations in limited areas The electron density of these parts diminished so that only shadows of the endoplasmic reticulum and ribosomes remained (Fig 24) Liver cells which had areas with extremely low electron density occurred while the cells otherwise, were not noticeably changed

Yttrium terbium, holmium, ytterbium (males and females) The material for this investigation was taken from parts of the liver in which no macroscopically observable necroses occurred In the cases investigated the liver cells showed a varying picture Cells which seemed to be normal and cells which showed various grades of injury were seen In a small number of cells the chromatin was distributed in the periphery of the nucleus or was clumped in the nucleus With the exception of a slight degree of enlargement the mitochondria showed no changes A dilatation of the cisternae of the endoplasmic reticulum was frequent in the liver cells investigated (Fig 25) The endoplasmic reticulum within limited areas sometimes was lacking in ribosomes These ribosomes then lay free in the cytoplasmic matrix in one section of the cell (Fig 25) In other cells electron dense clumps were seen which likewise consisted of aggregated ribosomes (Fig 26) In isolated liver cells the endoplasmic reticulum showed a diminished electron density within certain areas (compare with *Cerium* [males])

Discussion

The present investigation does not make pretensions to completeness where the question of toxic doses of lanthanons is involved This was not the aim of the experiments However the investigation gives certain suggestions in reference to the toxicity of the rare earth metals which can be valuable and which can merit attention primarily with thought to the scanty investigations which have been done in this area in the past According to the investigation done here the toxicity of the lanthanons is decreased with a rise in the atomic number This behavior has also been observed and emphasized by Kyker & Cress (1957) Even with intraperitoneal administration the toxicity of the lanthanons is shown to decrease with rising atomic numbers (Graca et al 1962) The doses used in the present investigation show that an appreciable difference prevails between the toxicities of the light and heavy lanthanons Yttrium is similar in many respects to the heavy lanthanons As far as the question of toxicity is concerned yttrium apparently seems to break away from this group

The present work shows that yttrium is more toxic than the heavy lanthanons

To compare the doses used in this investigation with those used in earlier toxicological investigations carried out on the rare earth metals is difficult because of the variation in experimental conditions The lanthanons are often administered by subcutaneous or intraperitoneal injection. This results in different resorption behaviors

In toxicological investigations of yttrium and other lanthanons on rats, Kyker & Cress (1957) used 3 5 mg cerium per kilogram for intravenous administration and obtained a mortality rate of 11 5 per cent after 21 days This differed appreciably from the results found here in relation to the question of dosing In the present investigation, a dose of 3 mg cerium per kilogram gave a significantly larger mortality in female rats, thus, within three days, 26 out of 49 rats died An explanation for this difference, or at least a contributory factor, could lie in the fact that different races were used in the two experiments Another possibility worth consideration is that the metallic salts were not completely identical, for example, in purity Kyker & Cress (1957) also investigated yttrium and holmium as chlorides using intravenous administration and gave a dose of 3 9 and 30 mg per kilogram body weight resp With these doses the authors obtained no mortalities In the present investigation, the doses of yttrium and holmium used were 9 and 40 mg, resp, per kilogram body weight With this dosage, several deaths occurred with both metals in female as well as male, rats (Table XII)

The present investigation shows that all of the lanthanons investigated exercise a lowering effect on the amount of glucose in the blood during the first days after intravenous administration. In female rats, this effect could be observed for three days and in male rats, for two days after the intravenous injection Thus this investigation clearly indicated that the rare earth metals are capable of lowering the blood sugar in rats Other details also merit attention The results give an indication that the effect on the blood glucose level commences more rapidly in female rats than in male rats In females, the blood glucose levels were lower than normal for all of the elements investigated as early as two hours after the injection This was not the case with males The experimental results show that the lowest blood glucose concentration prevails for the first two days after the administration in most cases However in female rats given cerium the lowest glucose levels occur during the second and third days (Fig 9 a)

The present experimental results confirm some earlier reports which state that the rare earth metals in a certain concentration are capable of lowering the level of glucose in the blood (Fischler & Roeckl 1938, Trapmann 1959, Snyder & Stephens 1961) Fischler & Roeckl (1938) made their observations on rabbits with lanthanum neodymium, praseodymium, yttrium, and cerit, which is a mixture of various lanthanons but mainly cerium, neodymium, and

lanthanum Snyder & Stephens (1961) observed the blood sugar lowering effect after intravenous administration of cerium to rats In contrast to the above mentioned works there is information which states that cerium lanthanum, neodymium, and praseodymium can produce a hyperglycemia in rabbits after intravenous administration (Mori 1931)

The regulation of the blood s constant concentration of glucose is accomplished by a combination of many different factors The hormonal function of the pancreas pituitary adrenals and thyroid among others plays a role In a discussion of the reasons why the rare earth metals produce hypoglycemia one might primarily consider the roles which the liver and pancreas play in carbohydrate metabolism

An increase of insulin in the blood produces hypoglycemia A cause of hypoglycemia is tumors of the pancreas producing insulin Lanthanons given intravenously to rats in toxic doses cause no changes in the pancreas (Magnusson unpublished observations) Furthermore, the lanthanons are not taken up to any great extent by the pancreas after intravenous administration (Ewaldsson & Magnusson 1963 a 1963 b) The proposal that removal of liver degeneration products should stimulate the pancreas to increased insulin production is considered to be without foundation (Fischler & Roeckl 1938) Thus it may be considered as highly unlikely that the hypoglycemia produced by the lanthanons is due to a disturbance in the pancreatic endocrine function

Instead the explanation of hypoglycemia should be sought in the liver with its key position in glucose metabolism Fischler & Roeckl (1938) thought the cause of hypoglycemia to be an inactivation of liver enzymes and suggested the designation Hypoglykamie durch Leberfermentlahmung It is known that liver glycogen plays an important role in the regulation of the glucose level of the blood Changes in the liver can give origin to hypoglycemia on the basis of insufficient glycogen formation in this organ (Bergstrand 1936) By chemical histological and electron microscopical methods it has been shown in the present work that the lanthanons investigated produced liver damage The glycogen level of the liver cells has been investigated histochemically An estimation of the amount of glycogen in the liver must always be done with care in respect to variations which exist under normal conditions In many cases—most clearly observable following the injection of cerium into female rats—a decrease of liver glycogen occurred In many cases the liver completely lacked glycogen In connection with this a lowered blood sugar concentration was generally established Hypoglycemia should therefore be justifiably carried back to acute liver damage produced by the rare earth metals One to one with this damage there follows an insufficient glycogen formation in the liver

The fact that liver damage can be accompanied by an insufficient formation of glycogen in the liver is shown by electron microscopic investigations According to Porter & Bruni (1959) some aminoazo dyes cause hypertrophy

of the smooth endoplasmic reticulum and at the same time, deprive the liver cells of their glycogen An analogous condition occurs with administration of the rare earth metals The present investigation shows the same changes in the endoplasmic reticulum of the liver cells (Fig 22) as those described by Porter & Bruni (1959) Probably the smooth endoplasmic reticulum takes part in glycogenesis and glycogenolysis (Porter & Bruni 1959) Thus according to this discussion the acute liver damage produced by the rare earth metals ought to be responsible for the insufficient glycogen formation in the liver which in its turn should cause hypoglycemia This confirms the work of Fischler & Roeckl (1938) who correlated liver glycogen reduction and liver lanthanon concentration It should also be kept in mind that a decreased appetite due to the intoxication plays a role in causing hypoglycemia However Lawrence et al (1959) have shown that a reduction in the food intake is not a causative factor in the production of the low glycogen content of lanthanon induced fatty liver

An increase of OCT in the blood is a sign of acute liver damage Very little information can be obtained from the literature on OCT determinations in connection with studies of rare earth metals Following intravenous administration of all of the lanthanons used in the present investigation increased OCT levels were found in the blood serum Sometimes the values were extremely high Thus the determination of the OCT level in the blood serum shows that the rare earth metals induce acute liver damage in rats The liver damage as indicated by the OCT values, begins soon after intravenous administration and reaches its maximum after 24 hours (Figs 10 a—b) However in female rats given cerium the liver damage developed somewhat more slowly and was not maximal until two to three days after administration

The liver damage induced by lanthanons has been studied morphologically in the present work Histological investigation shows that the rare earth metals cause regressive changes in the liver although different pictures are seen for the different elements

After the intravenous administration of cerium to female rats the liver shows a diffuse lesion in the form of a fatty degeneration. In this respect the investigation confirms the earlier known fact that certain lanthanons (lanthanum to samarium) produce fatty liver degeneration in female rats (Fischler & Roeckl 1938 Hara & Sato 1955 Kyker et al 1957 a, Neubert & Hoffmeister 1960 Snyder et al 1959 1960 a Snyder & Stephens 1961) In male rats however another picture was seen. In conformity with earlier work by Snyder et al (1959) the present investigation could demonstrate no fatty degeneration in the liver when the same doses as used in the female were used in the male On the other hand this investigation shows that cerium in male rats produces a slight damage in the liver with the same dose This lesion is not followed by fatty changes In the present work cerium was the only light

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lanthanon used In spite of this, the investigation can be said to strenghten the earlier known fact (Snyder et al 1959, 1960 a) that there are sex differences in relation to the toxicity of light lanthanons Female rats are more sensitive and thus demonstrate more serious liver damage than do male rats

The results show that the remaining lanthanons investigated—yttrium terbium, holmium, and ytterbium—produce morphological changes in the liver other than those caused by cerium These manifest themselves as focal necroses and are not followed by fatty changes Thus, these metals produce focal damage in the liver Even though only some of the heavy lanthanons were subjects of investigation, the remainder of the heavy lanthanons, in all probability, behave in the same manner as those elements investigated In this respect, yttrium is similar to the heavy lanthanons

In the focal necroses induced by the heavy lanthanons, multinucleated giant cells were observed Similar giant cells have also been seen in granulomatous peritonitis following the intraperitoneal administration of lanthanons in toxic doses to rats (Steffee 1959) It should be kept in mind that after intravenous and intraperitoneal administrations, the liver and the peritoneal cavity, respectively, contain a great amount of lanthanons No metabolism of the lanthanons takes place in the necrotic tissue of the liver The occurrence of multinucleated giant cells should probably be interpreted as a foreign body reaction to the metals

The present investigation shows that the rare earth metals, with respect to the morphological changes produced in the rat liver by toxic doses can be divided into two groups These coincide with the chemical division into light and heavy lanthanons The former show a sex difference while this is not the case with the latter The light lanthanons produce a diffuse fatty degeneration in the liver which, with identical dosing is demonstrable only in female rats The heavy lanthanons including yttrium, produce focal necroses in the liver which can be seen in female, as well as male, rats

Toxic lesions of the liver are usually distributed uniformly throughout the organ This fact, together with knowledge of the high concentration and even distribution of cerium in the liver (Aeberhardt et al 1961, Ewaldsson & Magnusson 1963 a), supports the opinion that the fatty degeneration is to be ascribed to a direct toxic effect of the metal on the liver cells

The remaining lanthanons investigated induced focal necroses in the liver It is more difficult to interpret these as expressions of a direct toxic effect on the liver cells Certain observations suggest that the necroses are developed because of a lowered blood supply With intravenous administration the rare earth metals lower the blood pressure (Steidle 1935 Haley et al 1961) The focal necroses were often observed in parts of the liver where the blood circulation under normal conditions can be poor, e g , the caudal lobe With continuing low blood pressure and reduced circulation, focal necroses occur in the

midzonal region of the liver lobuli (Himsworth 1948) The necroses had this localization in the present investigation Furthermore, the iron and fibrin staining indicated that the necroses were not of the hemolytic type These conditions suggest that heavy lanthanons primarily cause a lowered blood circulation and that thus, due to an insufficient nutrition of the liver cells, leads to the occurrence of focal necroses in the liver

It has been shown that in lanthanon induced fatty liver degeneration, the lipids consist of neutral fats (Snyder et al 1959) This is in agreement with the histochemical observations made in the present investigation

The electron microscopic investigations also suggested the occurrence of lipid droplets in the liver cells The development and localization of these droplets in the liver cell cytoplasm merit some discussion In the cases investigated, droplets have been observed which, at least sometimes, were surrounded by membranes These membranes can be of different origins According to the present investigation, the possibility that the contents of the dilatated cisternae of the endoplasmic reticulum were converted to lipid droplets (Figs 20, 21, 22, 23) can not be excluded Thus, the membranes surrounding the droplets might arise from the endoplasmic reticulum

The possibility that the lipid substance of the mitochondria can be increased is discussed by, among others, Neubert & Hoffmeister (1960) and Novikoff & Essner (1960) The former authors have shown, through the use of biochemical methods, that lanthanons (praseodymium, neodymium, and lanthanum) can produce an increase in the lipid content of the mitochondria in rats livers In the present investigation, mitochondria, whose ground substance had a conspicuous similarity to inclusions considered to be fat, were observed (Fig 19) Thus there is a possibility that the mitochondria are converted to lipid droplets

Injuries to cells manifest themselves as changes in the nuclei and cytoplasm This well known fact is also shown by the present investigation. Changes in the mitochondria and endoplasmic reticulum merit some attention In the present work, the mitochondria of the liver cells showed enlargement, changes in their ground substance, and lesions in their membranes Similar changes in the mitochondria of liver cells have been seen, e g, with hypoxia (Mölbert & Guerritore 1957) and following treatment with thyroxin (Schulz et al 1956)

A great number of biochemical reactions occur in the liver during metabolism These are catalyzed generally, by enzymes Novikoff (1961) reports that enzymes can be situated at different points within the same liver cell so that each biochemical reaction has its specific location According to the same author the mitochondria of the liver cells contain numerous enzymes which, above all, are oxidative in character Through the use of biochemical reactions (Snyder et al 1960 b, Glenn et al 1962), it has been shown that cerium injures the mitochondria of the liver cells. This fact has also been confirmed by

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Figure 11 Fatty liver from a female rat after intravenous administration of cerium.

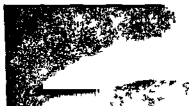


Figure 12 Focal necroses of a rat liver after intravenous administration of yttrium

the present electron microscopic investigation It can be established that cerium and probably other lanthanons can induce functional as well as morphological injury to the mitochondria of the rat liver

One feature of the present electron microscopic investigation was the occurrence of changes in the endoplasmic reticulum in both sexes with all of the lanthanons studied These changes were manifested primarily as dilatations of the cisternae of the endoplasmic reticulum with a dissociation of ribosomes Such changes have been observed e g with hypoxia (Bassi & Bernelli Zazzera 1957, Molbert & Guerritore 1957) and with carbon tetrachloride poisoning (Bassi 1960) In the latter investigation liver cells having areas which almost completely lacked endoplasmic reticulum occurred This picture is very reminiscent of that observed in the present investigation in male rats given cerium According to Bassi (1960), carbon tetrachloride injures first the endoplasmic reticulum and then the mitochondria The present investigation indicates that this could also be the case after administration of rare earth metals

Occasionally the dissociated ribosomes were aggregated into clumps This aggregation of ribosomes is possibly due to the fact that the rare earth metals bind nucleic acids (Hammarsten 1924 Hammarsten & Teorell 1928 Stern & Steinberg 1953) However, aggregations of ribosomes have also been observed in other cases e g viral hepatitis in mice (Svoboda et al 1962)

The liver microsomes which consist of the fragments of endoplasmic reticulum *in vitro* contain enzymes which are considered to take care of foreign compounds in the body (Brodie et al 1958) Experimental liver damage can be induced in many ways e g, with carbon tetrachloride phosphorus and ethionine Ethionine poisoning in rats causes a lowered function of the enzymes which are localized to the liver microsomes (Neubert 1957, Herken et al 1958 Neubert & Maibauer 1959) It is logical to suppose that this is also the case in liver damage induced by lanthanons This statement is supported partially by the fact that the microsomes contain lanthanons and partially by the fact that from a morphological viewpoint an injury to the endoplasmic reticulum (which corresponds to microsomes) occurs



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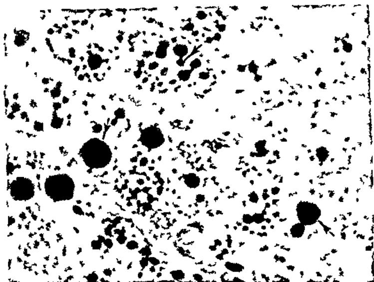
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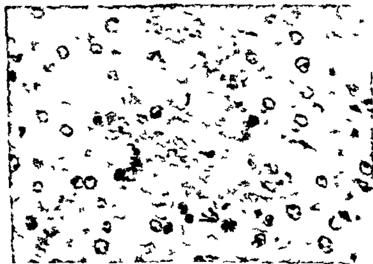
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F gure 13 Fatty degeneration in the liver of a female rat 3 days after intravenous ad m nistration of cerium Numerous fat droplets (at the arrows) in the liver cells Osmium fixative Tolu d ne blue 500 ×



F gure 14 Fatty liver degenerat on with necrosis in a female rat 3 days after intravenous adm n strat on of cerium Haemalum-eosin 500 ×



Figure 17 Parts of normal liver cells from a rat N=nucleus, m=mitochondria, ER= =endoplasmic reticulum mv=microvilli facing a sinusoid 12,000 ×



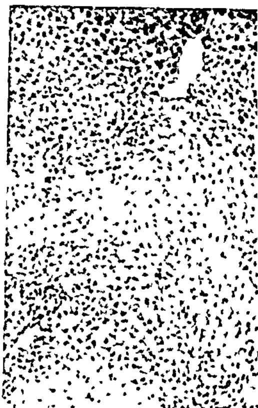
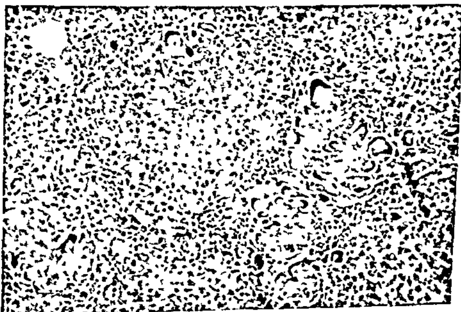
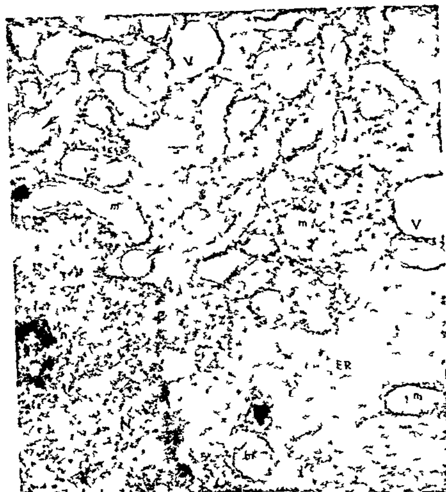


Figure 15 a Focal necroses in a rat liver 2 days after intravenous administration of yttrium Haemalum eosin 100 × — Figure 15 b Focal necroses in a rat liver 2 days after *intravenous administration of ytterbium Haemalum eosin 100 ×*



... of ytterbium
100 ×



F gu e 19 Pa of a l er cell 1 day af er ntra enous adm nis at on of cerium The nucleus N rregular n shape and the ch omat n is distribu ed along the ma g n In he ne ghbo hood of he nu leo us n the e a e dense clumps The nner s ructure of he m ochond a m s s rongly changed At the arrows two m ochondr a w h very l gh ma r ces a e seen wh ch are ery s m lar to the contents of the acuoles (V) A part of he ce l ER s occup ed by memb anes of he endoplasm c ret culum which lack r bosomes No e he d sarranged bosomes among the m ochondr a 17 000 ×



Figure 18 Part of a liver cell from a female rat 4 days after intravenous administration of cerium The nucleus contains lipid inclusions Note the reorganized granular endoplasmic reticulum (ER) in the cytoplasm 6000 ×



Figure 21 Part of a liver cell from a female rat 2 days after intravenous administration of cerium The endoplasmic reticulum shows dilated cisternae (ER) The vacuoles (V) are denser than after 1 day (compare Fig 19) The biliary capillary (BC) is filled with microvilli m=mitochondrion 16 000 ×

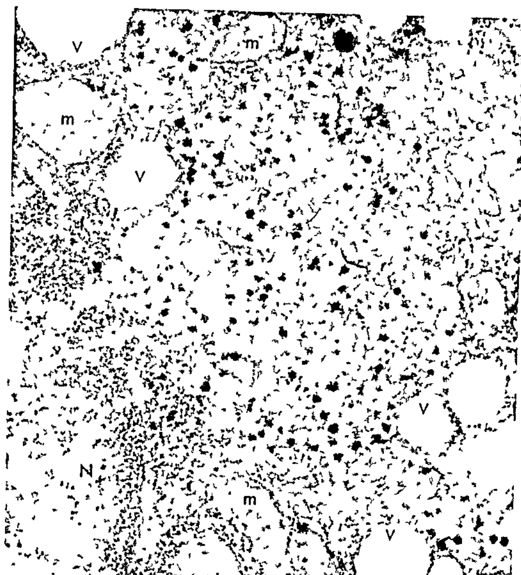


Figure 20 Part of a liver cell from a female rat 1 day after intravenous administration of cerium A part of the cytoplasm contains above all smooth endoplasmic reticulum Note the strongly electron dense clumps of aggregated ribosomes N=nucleus m=mitochondrion V=vacuoles 24 000 ×



Figure 23 Part of a liver cell from a female rat 3 days after intravenous administration of cerium. This cell contains osmiofilic inclusions surrounded by a single membrane (arrows). m=mitochondrion, ER=smooth endoplasmic reticulum. 18,000 ×



Figure 22 Part of a liver cell from a female rat 3 days after intravenous administration of cerium Part of the cell shows the extensive smooth endoplasmic reticulum intermingled with lipid droplets Bottom left and top right are lipopigment and lysosome like bodies m=mitochondrion N=nucleus *18 000* ×



Figure 23 Part of a liver cell from a female rat 3 days after intravenous administration of cerium This cell contains osmiofilic inclusions surrounded by a single membrane (arrows) m=mitochondrion, ER=smooth endoplasmic reticulum 18 000 ×

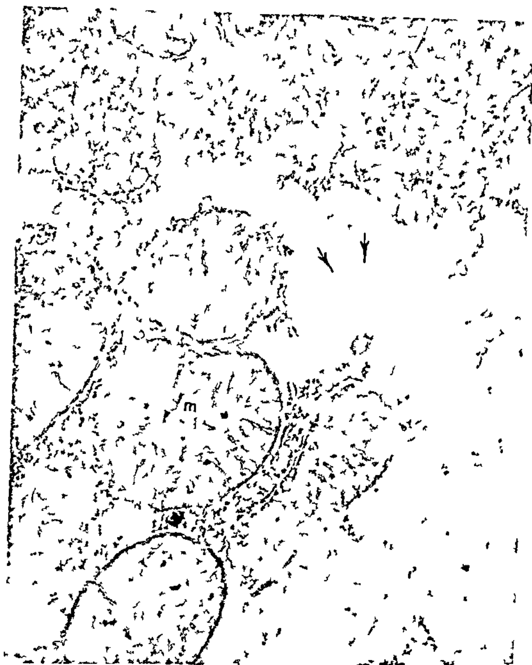


Figure 24 Part of a liver cell from a male rat 1 day after intravenous administration of cerium. The cytoplasm has an empty area. At the arrows membranes with very low contrast are seen. Note the slightly electron dense granules which appear to be shadows of ribosomes particularly seen in bottom right hand corner. m mitochondrion. 30 000 ×



F gu e 5 P f er el f om a male ra 12 hours af er n a enous admin trat on of ho n um The u pe ce hows d la ed cis ernae of he endopla m c ret culum Ar ow nd a e bo d m n ocl ond on mb m c obody 12 000 ×



Figure 26 Parts of two liver cells from a male rat 2 days after intravenous administration of ytterbium In the upper cell the endoplasmic reticulum is dilated and has partially lost its ribosomes The small electron dense clumps consist of aggregated ribosomes In the lower cell the ribosomes are arranged in rosettes Desmosomes (arrows) and a biliary capillary (BC) along the cell border are seen *m—mitochondrion mb microbody* 13 000 ×

CHAPTER VI

Summary and Conclusions

The aim of the present investigation was to study some aspects of the behavior of the rare earth metals in rats after intravenous administration Cerium, promethium, terbium, holmium, and ytterbium were chosen as representatives of the lanthanons In addition, yttrium, because of its similarity to the lanthanons, was investigated

The following questions were treated in the investigation, namely

1 The uptake of the lanthanons by the liver and kidneys and their excretion via the urine, bile, and gastro-intestinal tract,
2 The subcellular distribution of the lanthanons in the liver,
3 The acute toxic effect of the lanthanons on the liver

A literature survey of the rare earth metals is given In this, attention was drawn to the significance of the lanthanons from a biological viewpoint

1 as toxic elements
2 as helpful agents in medicine
3 as fission products

During the first three hours after intravenous administration, the blood concentration of the investigated lanthanons dropped with varying rates The blood concentration decreased most rapidly for Y^{91} and most slowly for Ho^{166} and Yb^{169}

The amount of investigated nuclides in the kidneys was a few per cent of the given dose during the first three hours after administration Y^{91} and Tb^{160} had the highest and Ho^{166} and Yb^{169} the lowest concentrations in the kidneys

Within a short time after the intravenous administration, the investigated lanthanons reached a high concentration in the liver The liver contained a maximum of 25—30 per cent of the given dose of Y^{91} Corresponding values for the remaining rare earth metals were 50 per cent or more The liver concentration diminished more rapidly for the heavy lanthanons including yttrium (Y^{91} Tb^{160} Ho^{166} and Yb^{169}) than for the light lanthanons (Ce^{144} and Pm^{147})

According to the present investigation, the excretion of the rare earth metals occurred in different ways, i e , via urine, bile, and gastro-intestinal wall During the first three hours the excretion via urine was greatest with Y^{91} and

least with Ho^{166} and Yb^{169} The investigation done here clearly shows that the *excretion via feces takes place in two ways* partly via bile and partly through the wall of the gastro intestinal tract Greater importance should be ascribed *to excretion via the wall of the* gastro-intestinal tract than has hitherto been done The gastric excretion was generally higher than the intestinal, at least *during the first hour after* administration

The subcellular distribution of the lanthanons in the liver was studied through differential centrifugation The investigation was done partly on a small number of animals (2) using many different time periods and partly on a larger number of animals (12) 24 hours after administration The former showed an uptake in the various cell fractions of all the investigated elements This, as a rule, was the case at all periods of time from two minutes to 192 hours after the intravenous administration The latter part of the investigation (24 hours after administration) included only Ce^{144} and Yb^{169} The nuclear fraction contained the lowest amount of both nuclides in female as well as male rats The largest amount of Ce^{144} as well as Yb^{169} occurred in the micro some fraction in female rats The male rats had the highest concentration of Ce^{144} in the supernatant and of Yb^{169} in the mitochondrial fraction and the supernatant A comparison between male and female rats shows that with Ce^{144} a statistically significant difference between the sexes occurs in all four liver cell fractions and with Yb^{169}, in all the liver cell fractions with the excep tion of the supernatant Between Ce^{144} and Yb^{169}, there exists a statistically significant difference in female rats in the mitochondrial and supernatant fractions and in male rats in all of the liver cell fractions except the supernatant

The acute toxic effect of the lanthanons on the liver was studied with non radioactive yttrium, cerium, terbium, holmium, and ytterbium An acute toxic effect on rats was obtained through the use of the following doses expressed as mg of metal per kilogram of body weight for the various lanthanons yttrium 9 mg, cerium 3 mg, terbium 35 mg, holmium 40 mg, and ytterbium 60 mg

The rare earth metals were seen to have a lowering effect on the glucose level in the blood during the first 3—4 days after the intravenous administration The lowest blood sugar levels were measured after injection of *cerium* into female rats

With the doses used, the lanthanons investigated induced acute *liver damage* This liver damage was studied partly by determinations of ornithine-carbamyl transferase (OCT) and partly by morphological *investigations*

During the first 3—4 days after intravenous administration increased OCT values occurred as a sign of acute liver damage *On many occasions* these were very high, e g, after the injection of cerium into female rats

The morphological picture *of the liver damage varied in the* lanthanons in-

vestigated Cerium, in female rats, caused a diffuse liver damage in the form of a fatty degeneration Cerium, in male rats, caused no fatty changes This result was reached using the same dose in both sexes All of the remaining lanthanons investigated—yttrium, terbium, holmium, and ytterbium—induced focal liver damage in the form of intralobular necroses in female as well as male rats The necroses were not followed by fatty changes These liver damages might explain the decrease in blood glucose

The electron microscopic investigation showed changes in the mitochondria and endoplasmic reticulum The mitochondria were enlarged, and their ground substance and cristae were changed Changes in the mitochondria were observed chiefly in female rats which had been administered cerium The changes in the endoplasmic reticulum were manifested as a dilatation of the cisternae and a loss of ribosomes The ribosomes often lay completely disarranged in the cytoplasmic matrix. At the same time, aggregations of ribosomes were seen Similar changes in the endoplasmic reticulum were seen with all of the investigated lanthanons in female as well as male rats

From the investigation done, the following conclusions may be drawn which most likely pertain to the entire lanthanon series, including yttrium, after intravenous administration to rats

1 The lanthanons are concentrated to a great extent in the liver
2 The lanthanons are excreted via urine and feces
3 The lanthanons' excretion via feces takes place in two ways, partly in the bile, and partly through the wall of the gastro-intestinal tract
4 The lanthanons are taken up by various cell organelles in the liver the cell nuclei, the mitochondria, and the microsomes
5 When given in a toxic dose the lanthanons exert a lowering effect on the blood glucose level
6 When given in a toxic dose, the lanthanons produce increased OCT values in blood serum as a sign of an acute liver damage
7 The light lanthanons, when the same toxic dose is used in both sexes, induce fatty degeneration of the liver in female rats but not in males
8 The heavy lanthanons including yttrium, when given in the same toxic doses to both sexes, induce focal necroses of the liver without fatty changes in both male and female rats.
9 The lanthanons in toxic doses, induce damage to the mitochondria and endoplasmic reticulum of the liver cells
10 In many respects, a sex difference exists for the light lanthanons but not for the heavy ones including yttrium

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EFFECT OF REST, WORK, AND RESERPINE TREATMENT

BY

OLLI CASTRÉN

MUNKSGAARD COPENHAGEN 1963

BERLINGSKA BOKTRYCKERIET
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SUND, REIDAR B and JENS SCHOU (From the Department of Pharmacology, University of Copenhagen, Denmark) *The Determination of Absorption Rates from Rat Muscles an Experimental Approach to Kinetic Descriptions* Acta pharmacol et toxicol, 1964, 21 (4), 313–325 (4 tables 2 figs, 17 ref)

Abstract

The rates of clearance (absorption) of mannitol (M), sucrose (S) inulin (I) and dextran (D), labelled with ^{14}C or ^{3}H after injection of 16 µl of solutions with tracer concentrations and NaCl added to isotonicity into the *m extensor quadriceps femoris* of rats are evaluated from determinations of the remaining radioactivity at the injection site after 5 15 and

remaining at the injection sites plotted against time give a curve (decrease of relative clearance

KÆMPE BENT (From the Section of Forensic Chemistry, Department of Pharmacology, University of Copenhagen Denmark) *Interfering Substances by Determination of Poisons in Autopsy Material I p-Hydroxyphenylehtanol* Acta pharmacol et toxicol, 1964, 21, (4), 326–332 (1 table 2 figs, 12 ref)

Abstract

The determin

KÆMPE BENT (From the Section of Foren
University of Copenhagen Denmark)
in Autopsy Material II Tyramine Ac
(2 tables 2 figs 13 ref)

Abstract

Tyramine was isolated and identified from human liver tissue stored
substance of similar
presence of tyram

LANGGÅRD, HANS (From the Department of Pharmacology, University of Copenhagen, Denmark) *Effects of Ascorbic Acid on Electrolyte Composition of the Skin of Mice* Acta pharmacol et toxicol, 1964 21, (4), 371-380 (6 tables, 0 figs, 17 ref)

Abstract

JÓHANNESSON TORKELL and L A WOO

Abstract

The analgesic effects of morphine and codeine were measured in rats by the hot plate method For morphine, ED50 was smaller after subcutaneous than after intraperitoneal, administration the reverse being true of codeine The analgesic effect was maximal 20

BRUNDIN T (From the Dep

Sweden) *Alkalosis*

Acta pharmacol et t

Abstract

A short circuit between artery and ve

SUND, REIDAR B and JENS SCHOU (From the Department of Pharmacology, University of Copenhagen Denmark) *Absorption of Atropine Anticholinergic Agents as Inhibitors of Absorption from Muscles* Acta pharmacol et toxicol, 1964, 21 (4), 339-346 (0 tables, 3 figs, 7 ref)

Abstract

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Abstract

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LANGGÅRD HANS (From the Department of Pharmacology, University of Copenhagen, Denmark) *Effects of Adrenalectomy on Connective Tissue Electrolytes in Mice* Acta pharmacol et toxicol, 1964, 21, (4) 365-370 (2 tables, 0 figs, 13 ref)

Abstract

Untreated male mice and mice pretreated with oestradiol were adrenalectomized

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PREFACE

While working as Assistant Physician at the Pharmacological Department of the University of Turku Professor Aimo Pekkarinen M D Head of the Department gave me the incentive to the present investigation Professor Pekkarinen s extensive knowledge of the methods for determining noradrenaline and adrenaline and of the literature concerning these substances which he has generously shared with me have greatly facilitated my studies My sincere thanks are due to him for his never failing interest in my work and for permitting his staff to assist me in various ways

The majority of the samples were obtained from the Department of Obstetrics and Gynecology of the University of Turku The late Professor Sakari Parviainen M D Head of the Department at the time of starting my research was widely known as an ardent investigator of toxemia of pregnancy and my esteemed teacher of obstetrics and gynecology He followed my investigation with keen interest for which I am greatly thankful

To Docent Keijo Soiva M D Acting Professor of Obstetrics and Gynecology of the University of Turku I extend my thanks for taking part in instructive discussions on the clinical aspects of my study

I am greatly indebted also to Docent Lauri Rauramo M D former Acting Professor of Obstetrics and Gynecology of the University of Turku and to Dr Robert Hasselblatt M D Head of the Maternity Hospital of the City of Turku for allowing me to collect the samples in their hospitals

Likewise I extend my thanks to the maternity nurses of the mentioned hospitals and to those of the Maternity Welfare Centres of the City of Turku who have helped me in collecting the samples

Mrs Aili Ryynänen M A Librarian of the Medical Library of the University of Turku and her staff have assisted me in obtaining the literature required for my study The staff of the Pharmacological Department have aided me with determinations of the samples over a period of several years Miss Alice M Eager has translated my paper into English and Mr T Pasanen M A performed the statistical treatment To all of these I extend my sincere gratitude

Financial aid was granted for the initial part of my study by the Mater Foundation

Turku October 1963

Olli Castren

INTRODUCTION

During pregnancy, the female organism adapts itself to reproduction, nutrition and metabolism of the fetus The homeostasis of the maternal organism must be maintained under circumstances in which the body weight increases by one fifth to one-eighth and the growth of the uterus and fetus influences mobility, and also the function of the organs, especially of the abdominal cavity Numerous metabolic changes are characteristic of pregnancy e g, formation of estrogens progesterone and gonadotropic hormones increases greatly, the plasma content of free 17 hydroxycortico steroids duplicates and combines with transcortin, the amount of water retaining steroids increase fluid and salt retention occurs in the tissues, and alterations in the blood circulation take place

It is therefore interesting to study the balance of the sympathetic nervous system and its connection with the increased steroid hormone balance during pregnancy both of which are needed for maintenance of normal blood pressure and internal homeostasis

The changes observed in women during normal pregnancy are partly required for protection of both fetus and mother However, the increased functions are evidently too strenuous in certain individuals, and the changes regulating the internal homeostasis of the female organism do not give sufficient protection and a condition termed toxemia of pregnancy results The symptoms of this disease are excessive increase in body weight, elevated systolic and diastolic blood pressure, and proteinuria

The blood pressure rises even after intravenous infusion of small doses of noradrenaline and adrenaline and the adrenaline causes significant metabolic changes (HELVE & PEKKARINEN 1952, PEKKARINEN & ARO 1952) Moreover it has been observed that, after infusion of noradrenaline during toxemic pregnancy, the increase in blood pressure is greater than during normal pregnancy (RAAB *et al* 1956) From a clinical point of view, the sympathetic nervous system often seems to dominate the neurovegetative balance in toxemic pregnancy (RAAB 1953) This is evidenced by the results of for instance, the cold pressor test and Schellong's orthostatic test (HAUSER 1960)

It is well known that the blood contains very small amounts of adrenaline and noradrenaline (PEKKARINEN 1948a, LUND 1950, 1951, v EULER 1956, VALK & PRICE 1956, WEIL-MALHERBE & BONE 1957, MANGER *et al* 1959, VENDSALU 1960) The urine contains larger amounts of these substances (PEKKARINEN 1954 PEKKARINEN & PITKÄNEN 1952a, 1955, KARKI 1956,

PITKÄNEN 1956 PEKKARINEN *et al* 1961) and the tissues still larger amounts (v. EULER 1946 PEKKARINEN & PITKÄNEN 1952 b v. EULER 1956) Also large amounts of adrenaline (PEKKARINEN 1948 a) and noradrenaline (LUND 1951) disappear rapidly from the blood circulation The uptake of this substances from the blood into the tissues takes place in all the capillaries (GOODALL *et al* 1959 AXELROD *et al* 1959 HERTTING *et al* 1961 a) and may be regulated by drugs (HERTTING *et al* 1961 b)

In toxemic pregnancy the noradrenaline and adrenaline content is not increased in the plasma (MANGER *et al* 1959 HOCHULI 1960) nor in the urine as determined by biological methods (BURN 1953) However these substances together with the changes observed in the steroid balance may contribute to the development of toxemic pregnancy

Rest ameliorates the symptoms of toxemic pregnancy and both bodily and mental strain impairs the condition In healthy persons rest decreases (v. EULER *et al* 1955 b) and physical exercise increases the excretion of noradrenaline and adrenaline (KÄRKI 1956) Mental strain again raises the urinary excretion particularly of adrenaline (PEKKARINEN *et al* 1961) It therefore seemed appropriate to study to what extent normal and toxemic pregnancy as such and especially exertion and rest affect the output of these two substances under the conditions mentioned

Moreover the 24 hour variation in the excretion of noradrenaline and adrenaline illustrates the effect of rest and daily activity Hence determination of the day and night excretion of noradrenaline and adrenaline in the urine each separately in late normal and toxemic pregnancies is of interest

Treatment of toxemic pregnancy in the present day is based on promotion of saluresis and diuresis by peroral administration of saluretic diuretics and antihypertensive agents such as reserpine Administration of large doses of reserpine evidently decreases especially the noradrenaline excretion in the urine (CARLSSON *et al* 1959 a) since tests on animals have revealed that the drug decreases the noradrenaline content in the tissues (CARLSSON *et al* 1957 PAASONEN & KRAYER 1957 PEKKARINEN *et al* 1958)

If the favourable effect of reserpine is based on its decreasing action on the noradrenaline and adrenaline content in the tissues and if this effect were to be revealed by the urinary excretion of noradrenaline in toxemic patients this would point to the possibility that noradrenaline a factor which regulates the vascular tone is partly responsible for the development of toxemia of pregnancy

In this investigation attempts have therefore been made to clarify the effect of reserpine on the noradrenaline and adrenaline excretion in patients resting in bed owing to toxemic pregnancy of various degree as well as the action of the drug on the excretion of the two substances in pregnant women exposed to the physical exertion of normal daily life

PREVIOUS INVESTIGATIONS

URINARY EXCRETION OF NORADRENALINE AND ADRENALINE

Normal condition

Fetus and newborn infants

During the *fetal stage* the adrenal gland contains mainly noradrenaline (HOKFELT 1951 SHEPHERD & WEST 1951 a b NIEMINEVA & PEKKARINEN 1952) However at an early stage of the fetal development the gland is able to methylate noradrenaline into adrenaline (NIEMINEVA & PEKKARINEN 1952 GREENBERG & LIND 1961) As the fetus grows the secretion of adrenaline increases and at the time of parturition the noradrenalin and adrenaline level in the adrenal gland is about the same as in adult (FRANKO 1956)

Small amounts of noradrenaline and adrenaline are excreted in the urine of *new born infants* (Table 1) The average excretion of noradrenaline varies between 0 6 and 1 5 µg/24 hours and that of adrenaline between 0 12 and 0 29 µg/24 hours (ZEISEL & KUSCHKE 1959 GREENBERG & GARDNER 1960 CASTREN *et al* 1961 1963) The excretion of adrenaline in the new born 0 05 to 0 12 µg/24 hrs/kg of body weight (GREENBERG *et al* 1960 STERN *et al* 1961 CASTRÉN *et al* 1963) corresponds to that in adults During the three first days of life the average excretion of adrenaline is greatest on the first day and decreases slightly on the following days The excretion of noradrenaline again is 0 38 µg/24 hours on the first day and increases gradually being 0 71 µg/24 hours on the third day (CASTRÉN *et al* 1963)

Healthy persons

Between the age of seven and sixteen (KARKI 1956) the noradrenaline level rises significantly per kilogramme of body weight this is evidently due to the considerable activity at that age whereas according to ZEISEL & KUSCHKE (1959) the excretion reaches the maximum in the first ten years of life The adrenaline excretion in childhood does not differ per kilogramme of body weight from that in other periods of life nor is a difference observed between boys and girls in this respect (KARKI 1956)

Table 1

Urinary excretion of noradrenaline and adrenaline in healthy persons (μg/24 hours)

References	n	Noradrenaline	Adrenaline	Method	Remarks
a) Newborn					
Zeisel & Kuschke 1959	10	0.90	0.12	fluor	
Greenberg & Gardner 1960	21	1.51	0.29	"	
Greenberg *et al* 1960	7	0.37	0.12	fluor	μg/kg/24 hours
Greenberg *et al* 1960	18	0.26	0.05	"	"
Steen et al 1961	21	0.43	0.08	"	"
Castrén *et al* 1963	40	0.57	0.23	biol	
b) Children					
Burn 1953	9	10.0		biol	1.4—10 years
Burn 1953	9	18.0		" (hydrol.)	"
Farqukar *et al* 1956	5	8.4	3.6	fluor	11 months ♂
Farqukar *et al* 1956	5	11.5	7.2	" (hydrol.)	"
Karki 1956	30	6.4	1.3	biol	1.5—6 years ♂
Karki 1956	12	4.5	1.3	"	" ♀
Karki 1956	30	16.0	3.0	"	7—16 years ♂
Karki 1956	20	12.3	2.4	"	" ♀
Zeisel & Kuschke 1959	10	8.0	1.2	fluor	2—3 years
Zeisel & Kuschke 1959	10	12.8	2.9	"	11—16 years
c) Adults					
v. Euler & Hellner 1951	20	29.0	11.5	biol (hydrol.)	
Burn 1953	10	44.0		biol	
v. Euler *et al* 1954c	15	27.4	5.6	"	
v. Euler *et al* 1955a	15	27.0	4.3	"	
Pekkarinen & Pitkanen 1955	17	81		fluor	
Pitkanen 1956	16	54		"	
de Schaepdryver *1958*	17	38.7	9.3	"	μg/l
Pekkarinen *et al* 1961	44	17.9	5.2	biol	
v. Euler *et al* 1955b	10	17.7	6.7	fluor	resting
Sundin 1956	10	16.5	4.8	"	"
Halme *et al* 1957	63	24.3	4.4	"	"
Karki 1956	48	25.7	5.9	biol	men
Pekkarinen *et al* 1961	22	21.6	6.8	"	"
Halme et al 1957	36	26.4	4.8	"	" , resting
Karki 1956	38	22.9	4.2	"	women
Pekkarinen et al 1961	26	15.3	3.5	"	"
Halme *et al* 1957	27	21.7	3.7	"	" , *resting*

Biologically active catechols are excreted in the urine of *healthy adults* (Table 1) in amounts of 100 to 150 μg/24 hours as determined by the cat s blood pressure method (HOLTZ *et al* 1947) In healthy male students the average 24 hour excretion of noradrenaline was 29 μg after hydrolysis and that of adrenaline 11 5 μg (v EULER & HELLNER 1951) and correspondingly the excretion of free noradrenaline 27 μg/24 hours and that of free adrenaline 4 3 μg/24 hours (v EULER *et al* 1955a) As evidenced by the fluorimetric methods the 24 hour excretion of noradrenaline in healthy people was 81 μg (PEKKARINEN 1954 PEKKARINEN & PITKANEN 1955) but at a later date using the same methods with addition of butanol extraction the average noradrenaline excretion was 54 μg/24 hours and that of adrenaline in the total amount of biologically active catechol 33 per cent (PITKANEN 1956) Differentiation of the noradrenaline and adrenaline amounts by the fluorimetric method showed that the former was 36 7 μg litre and the latter 9 3 μg/litre (DE SCHAEPDRYVER 1958) The excretion of biologically active free noradrenaline was 25 μg/24 hours in 17 to 59 year old men and that in women of the same age group 23 μg/24 hours the adrenaline excretion was correspondingly 6 μg/24 hours in men and 4 μg/24 hours in women (KARKI 1956) Before major surgical operations the average noradrenaline excretion in women was 21 7 μg/24 hours and the adrenaline excretion 3 7 μg/24 hours (HALME *et al* 1957) The noradrenaline excretion was 15 3 μg 24 hours and that of adrenaline 3 5 μg/24 hours in female students (PEKKARINEN *et al* 1961) Thus in normotensive persons the biologically determined urinary excretion of free noradrenaline does not generally exceed 50 μg/24 hours (GOLDENBERG *et al* 1954 v EULER *et al* 1954) Determination by the fluorimetric methods revealed that the excretion of noradrenaline varies between 20 and 60 μg/24 hours and that of adrenaline between 10 and 30 μg/24 hours (GOODALL & BOGDANOFF 1961)

In *elderly persons* after the age of 60 the urinary excretion of noradrenaline and adrenaline is again slightly decreased (KARKI 1956)

Diurnal rhythm

The excretion of noradrenaline and adrenaline is highly influenced by the activity of the organism The average day time excretion of noradrenaline in the urine of normal persons is 2 0 to 5 5 times greater than the night time excretion (Table 2) and that of adrenaline 2 5 to 10 times greater (v EULER *et al* 1955a ELMADJIAN *et al* 1956c KARKI 1956 ELMADJIAN *et al* 1957 v EULER & LISHAJKO 1959 JANUSZEWICZ & WOCIAL 1960) In patients confined to bed there was no difference between the day and night excretion of adrenaline but the day time excretion of noradrenaline was 1 5 to 3 times greater (v EULER *et al* 1955a ELMADJIAN *et al* 1956c JANUSZEWICZ & WOCIAL 1960) The amount of noradre

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Greenberg *et al.* 1960	18	0.26	0.05	„	„
Stern *et al.* 1961	21	0.43	0.08	„	„
Castrén *et al.* 1963	40	0.57	0.23	biol	
b) Children					
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de Schaepdryver 1958	17	36.7	9.3	„	μg/l
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Kärki 1956	38	22.9	4.2	„	women
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In elderly persons, after the age of 60 the urinary excretion of noradrenaline and adrenaline is again slightly decreased (KÄRKI 1956).

Diurnal rhythm

The excretion of noradrenaline and adrenaline is highly influenced by the activity of the *organism*. The average day time excretion of *noradrenaline in the urine* of normal persons is 2.0 to 5.5 times greater than the night time excretion (Table 2) and that of adrenaline 2.5 to 10 times greater (v. EULER *et al* 1955a, ELMADJIAN *et al* 1956c, KÄRKI 1956, ELMADJIAN *et al* 1957, v. EULER & LISHAJKO 1959, JANUSZEWICZ & WOCIAL 1960). In patients confined to bed there was no difference between the day and night excretion of adrenaline, but the day time excretion of noradrenaline was 1.5 to 3 times greater (v. EULER *et al* 1955a, ELMADJIAN *et al* 1956c, JANUSZEWICZ & WOCIAL 1960). The amount of noradre

Table 2

Diurnal rhythm of urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Night	Noradrenaline Day	Adrenaline Night	Adrenaline Day	Method	Remarks
BURN 1953	12	0.25	1.50			biol	
BURN 1953	12	0.71	1.17			,, (hydrol.)	
v. EULER *et al.* 1955a	15	0.59	1.38	0.07	0.26	biol	
ELMADJIAN *et al.* 1957	6	1.20	2.40	0.02	0.21	,,	
v. EULER & LISHAJKO 1959	5	0.56	1.50	0.12	0.63	fluor	
JANUSZEWICZ & WOCIAL 1960	10	0.28	1.53	0.08	0.39	,,	
KARKI 1956	18	0.71	1.50	0.14	0.39	biol	men
KARKI 1956	18	0.62	1.35	0.09	0.24	,,	women
v. EULER *et al.* 1955b	10	0.51	0.74	0.23	0.28	,,	resting
ELMADJIAN *et al.* 1956c	7	1.20	2.70	0.04	0.33	,, (hydrol.)	,,
SUNDIN 1956	10	0.51	0.74	0.15	0.28	biol	,,
JANUSZEWICZ & WOCIAL 1960	10	0.30	0.94	0.12	0.14	fluor	,,
JANUSZEWICZ & WOCIAL 1960	10	1.28	0.88	0.39	0.31	,,	nightwork
JANUSZEWICZ & WOCIAL 1960	10	0.85	0.87	0.16	0.18	,,	blind
BURN 1953	9	0.17	0.25			biol	infants
KARKI 1956	4	0.33	0.69	0.04	0.12	,,	,,

naline and adrenaline excreted in persons doing night work was similar to that in persons working in the day-time, but the amounts excreted during the rest period were twice those excreted in persons with normal shift work (JANUSZEWICZ & WOCIAL 1960). No difference between the day and night time excretion of these substances was noted in blind people (JANUSZEWICZ & WOCIAL 1960).

Body posture

The position of the body affects the excretion of noradrenaline and adrenaline (Table 3). When the person tested is tilted from a recumbent position to an angle of 50 to 75°, head up, the excretion of noradrenaline increases 2.8 to 3.5 times and that of adrenaline 1.6 to 5 times (v. EULER *et al.* 1955 b; SUNDIN 1956, 1958). The noradrenaline and adrenaline excretion decreases in test persons, whether fasting or allowed food, but tilting after this period increases the excretion of noradrenaline 6 times and that of adrenaline 5 times (SUNDIN 1958). The position of the body does not alter the amount of noradrenaline or adrenaline excreted in hypertensives (SUNDIN 1958). In newborn infants, the excretion of both noradrenaline and adrenaline increases to about 2.5 times the normal (GREENBERG *et al.*

Table 3

Effect of body posture on urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Recumbent	Noradrenaline Tilting 75°	Adrenaline Recumbent	Adrenaline Tilting 75°	Method	Remarks
v. Euler *et al.* 1955 b	15	0.65	1.85	0.24	0.75	biol.	
Sundin 1956	22	0.59	2.05	0.11	0.57	„	
Sundin 1958	10	0.99	3.35	0.27	0.44	„	
v. Euler & Franksson 1957	10	0.75	1.46	0.02	0.03	„	after adrenalectomy
Sundin 1958	4	0.45	2.73	0.09	0.52	„	eating before test
Sundin 1958	18	0.62	1.90	0.11	0.58	„	fasting before test
Sundin 1958	10	0.56	0.53	0.47	0.52	„	hypertension

1960). After bilateral adrenalectomy the noradrenaline and adrenaline excretion increases to the double on tilting to an angle of 75°, head up (v. Euler & Franksson 1957).

Effect of physical exercise and psychic tension on the excretion of noradrenaline and adrenaline

Physical exercise

In spite of the amount of free noradrenaline and adrenaline excreted in the urine being small, 0.5 to 5 per cent, it may serve as a basis for

Table 4

Effect of physical exercise on urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Controls	Noradrenaline Competition	Adrenaline Controls	Adrenaline Competition	Method	Remarks
v. Euler & Hellner 1952	15		12—30		1.2—6	biol.	
Karki 1956	10	1.8	4—11	0.25	2—6	(hydrol.) biol.	Marathon runners
Karki 1956	5	1.8	14.6	0.25	1.8	„	wood cutters
Elmadjian *et al.* 1957	20	2.7	15.3	0.36	0.95	„	hockey players (µg/100 mg creatinine)
Elmadjian *et al.* 1957	3	16.3	13.3	0.9	0.67	„	amateur boxers
McGowdall & Berman 1960	9	1.56	2.45	0.86	2.27	„	centrifugation
Takahashi 1961	2	0.74	2.54	0.38	0.76	(hydrol.) biol.	bicycle ergometer (µg/100 mg

Table 2

Diurnal rhythm of urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Night	Noradrenaline Day	Adrenaline Night	Adrenaline Day	Method	Remarks
BURN 1953	12	0.25	1.50			biol	
BURN 1953	12	0.71	1.17			„ (hydrol.)	
v. EULER *et al.* 1955a	15	0.59	1.38	0.07	0.26	biol	
ELMADJIAN *et al.* 1957	6	1.20	2.40	0.02	0.21	„	
v. EULER & LISHAJKO 1959	5	0.56	1.30	0.12	0.63	fluor	
JANUSZEWICZ & WOCIAL 1960	10	0.28	1.53	0.08	0.39	„	
KARKI 1956	18	0.71	1.50	0.14	0.39	biol	men
KARKI 1956	18	0.62	1.35	0.09	0.24	„	women
v. EULER *et al.* 1955b	10	0.51	0.74	0.23	0.28	„	resting
ELMADJIAN *et al.* 1956c	7	1.20	2.70	0.04	0.33	„ (hydrol.)	„
SUNDIN 1956	10	0.51	0.74	0.15	0.28	biol	„
JANUSZEWICZ & WOCIAL 1960	10	0.30	0.94	0.12	0.14	fluor	„
JANUSZEWICZ & WOCIAL 1960	10	1.28	0.88	0.39	0.31	„	nightwork
JANUSZEWICZ & WOCIAL 1960	10	0.65	0.87	0.16	0.18	„	blind
BURN 1953	9	0.17	0.25			biol	infants
KARKI 1956	4	0.33	0.69	0.04	0.12	„	„

naline and adrenaline excreted in persons doing night work was similar to that in persons working in the day time but the amounts excreted during the rest period were twice those excreted in persons with normal shift work (JANUSZEWICZ & WOCIAL 1960). No difference between the day and night time excretion of these substances was noted in blind people (JANUSZEWICZ & WOCIAL 1960).

Body posture

The position of the body affects the excretion of noradrenaline and adrenaline (Table 3). When the person tested is tilted from a recumbent position to an angle of 50 to 75°, head up, the excretion of noradrenaline increases 2.8 to 3.5 times and that of adrenaline 1.6 to 5 times (v. EULER *et al.* 1955 b; SUNDIN 1956, 1958). The noradrenaline and adrenaline excretion decreases in test persons, whether fasting or allowed food, but tilting after this period increases the excretion of noradrenaline 6 times and that of adrenaline 5 times (SUNDIN 1958). The position of the body does not alter the amount of noradrenaline or adrenaline excreted in hypertensives (SUNDIN 1958). In newborn infants, the excretion of both noradrenaline and adrenaline increases to about 2.5 times the normal (GREENBERG *et al.*

Table 3

Effect of body posture on urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Recumbent	Noradrenaline Tilting 75°	Adrenaline Recumbent	Adrenaline Tilting 75°	Method	Remarks
v. EULER *et al.* 1955 *b*	15	0 65	1 85	0 24	0 75	biol.	
SUNDIN 1956	22	0 59	2 05	0 11	0 57	„	
SUNDIN 1958	10	0 99	3 35	0 27	0 44	„	
v. EULER & FRANKSSON 1957	10	0 73	1 46	0 0?	0 03	„	after adrenalectomy
SUNDIN 1958	4	0 45	2 73	0 09	0 52	„	eating before test
SUNDIN 1958	18	0 62	1 90	0 11	0 58	„	fasting before test
SUNDIN 1958	10	0 56	0 53	0 47	0 52	„	hypertension

1960). After bilateral adrenalectomy the noradrenaline and adrenaline excretion increases to the double on tilting to an angle of 75°, head up (v. EULER & FRANKSSON 1957).

Effect of physical exercise and psychic tension on the excretion of noradrenaline and adrenaline

Physical exercise

In spite of the amount of free noradrenaline and adrenaline excreted in the urine being small, 0 5 to 5 per cent, it may serve as a basis for

Table 4

Effect of physical exercise on urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Controls	Noradrenaline Competition	Adrenaline Controls	Adrenaline Competition	Method	Remarks
v. EULER & HELLNER 1952	15		12—30		1 2—6	biol.	
KARKI 1956	10	1 8	4—11	0 25	2—6	(hydrol.) biol.	Marathon runners
KARKI 1956	5	1 8	14 6	0 25	1 8	„	wood cutters
ELMADJIAN *et al.* 1957	20	2 7	15 3	0 36	0 95	„	hockey players
ELMADJIAN *et al.* 1957	3	16 3	13 3	0 9	0 63	„	(µg/100 mg creatinine) amateur boxers
MCGOODALL & BERMAN 1960	9	1.56	2 45	0 56	2 27	„ centrifugation	
TAKAHASHI 1961	3	0 74	2 54	0 38	0 70	(hydrol.) biol.	bicycle ergometer (µg/100 mg

evaluation of the excretion under different conditions. Physical activity augments the urosympathin excretion (Holtz *et al.* 1947). During *muscular exercise*, such as short skiing competitions, training for sports for 45 *to 70* minutes, and ice hockey matches, the excretion of noradrenaline increases 3 to 20 times (Table 4), and that of adrenaline 3 to 10 times (v. Euler & Hellner 1952, Karki 1956, Elmadjian *et al.* 1957), and during heavy *muscular work*, Marathon runs, and wood cutting in exceptional cases about 17 and 25 times, respectively (Karki 1956). Eosinophilia develops simultaneously with the rise in noradrenaline and adrenaline excretion due

Table 5

Effect of psychic tension on urinary excretion of noradrenaline and adrenaline (μg/hour)

References	n	Noradrenaline Controls	Noradrenaline Stress	Adrenaline Controls	Adrenaline Stress	Method	Remarks
v. Euler & Lundberg *1954*	14	1.4	1.6	0.4	1.4	biol. (hydrol.)	flying as passenger
v. Euler & Lundberg 1954	13	1.14	2.34	0.31	1.14	biol. (hydrol.)	flying as pilot
Franksson *et al.* 1954	6	0.63	0.96	0.13	0.20	biol.	*operation*
Elmadjian *et al.* 1956c	4	2.7	3.8	0.21	0.55	„	Hoagland Werthessen pursuitmeter
Elmadjian *et al.* 1957	2	3.9	4.3	0.5	1.1	„	reserve hockey players
Elmadjian *et al.* 1957	11	2.3	2.6	0.27	0.50	„	psychiatric patients
Halme *et al.* 1957	83	1.01	2.46	0.18	0.56	„	operation, in whole material
Halme *et al.* 1957	44	1.1	2.97	0.20	0.71	„	operation, in men
Halme *et al.* 1957	36	0.90	1.62	0.15	0.41	„	operation, in women
Zuidema *et al.* 1957	3	2.18	5.9	1.64	3.87		G tolerance, anxiety reaction
Zuidema *et al.* 1957	3	3.89	11.9	0.89	3.03	„	G tolerance, anger reaction
v. Euler *et al.* 1959b	10	0.92	1.25	0.41	0.70	„	selected exciting movies
Goodall & Berman 1960	9	1.5	1.6	0.86	1.93	„	mock centrifugation
Mendelson *et al.* 1960	10	1.03	2.09	0.28	0.56	fluor.	sensory deprivation
Pekkarinen *et al.* 1961	189	0.75	0.81	0.22	0.85	biol.	matriculation examination
Frankenhaeuser & Post 1962	16	1.2	1.43	0.5	0.91	fluor.	psychological tasks

to physical exercise (TAKAHASHI 1961) Muscular exercise on a bicycle ergo meter for 4 to 9 minutes, working at 25 kgm/second, causes a threefold increase in the noradrenaline content in the plasma, and a fourfold increase in the adrenaline content (di IANNACCONE *et al* 1961)

Psychic tension

Psychic tension (Table 5) does not as a rule affect the excretion of noradrenaline to any extent, but several factors such as travel by air (v EULER & LUNDBERG 1954), exciting films (v EULER *et al* 1959b) and staff interviews for psychiatric patients (ELMADJIAN *et al* 1957), and psychotherapeutic sessions for the patient and the therapist (ELMADJIAN *et al* 1958) augment the excretion of adrenaline to the double Mental strain corresponding to that connected with matriculation or to examinations at selection courses for gaining admission to the Faculty of Medicine, also causes a fourfold increase in the average excretion of adrenaline the greatest excretion observed was 100 8 µg/day (PEKKARINEN *et al* 1961) In test animals, fear increases the excretion of both noradrenaline and adrenaline (PEKKARINEN *et al* 1960b) and the plasma content of these substances (MASON *et al* 1961)

Effect of some pathological conditions on the excretion of noradrenaline and adrenaline

Cardiovascular diseases

In *congestive heart failure* (Table 6) the average excretion of noradrenaline is mainly normal but a slight rise occurs on medium impairment of the condition and a decrease when the condition becomes severe (PEKKARINEN *et al* 1960a)

The urinary excretion of noradrenaline in *coronary thrombosis* (Table 6) increases owing to activation of the sympathetic nervous system due to shock and pain but does not however, often exceed 100 µg/24 hours, the average excretion of adrenaline is not increased (FORSSMAN *et al* 1952, FORSSMAN 1954) The adrenaline content in the plasma rises to three times the normal and that of noradrenaline to almost twice the normal, this content is correlated to the serum transaminase (SGOT) which illustrates the width of the necrosis (GAZES *et al* 1959)

Mild physical exercise does not change the noradrenaline and adrenaline content in the plasma of man, but raises the level in patients with angina pectoris to twice the normal (GAZES *et al* 1959) In animals coronary occlusion increases the noradrenaline content of plasma seven times

evaluation of the excretion under different conditions. Physical activity augments the urosympathin excretion (HOLTZ *et al.* 1947). During muscular exercise, such as short skiing competitions, training for sports for 45 to 70 minutes, and ice hockey matches, the excretion of noradrenaline increases 3 to 20 times (Table 4), and that of adrenaline 3 to 10 times (v. EULER & HELLNER 1952, KARKI 1956, ELMADJIAN *et al.* 1957), and during heavy muscular work, Marathon runs and wood cutting, in exceptional cases about 17 and 25 times, respectively (KARKI 1956). Eosinophilia develops simultaneously with the rise in noradrenaline and adrenaline excretion due

Table 5

Effect of psychic tension on urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Controls	Noradrenaline Stress	Adrenaline Controls	Adrenaline Stress	Method	Remarks
v. EULER & LUNDBERG 1954	14	1.4	1.6	0.4	1.4	biol (hydrol.)	flying as passenger
v. EULER & LUNDBERG 1954	13	1.14	2.34	0.31	1.14	biol (hydrol.)	flying as pilot
FRANKSSON *et al.* 1954	6	0.63	0.96	0.13	0.20	biol	operation
ELMADJIAN *et al.* 1956c	4	2.7	3.8	0.21	0.55	"	Hoagland-Werthessen pursuitmeter
ELMADJIAN *et al.* 1957	2	3.9	4.3	0.5	1.1		reserve hockey players
ELMADJIAN *et al.* 1957	11	2.3	2.6	0.27	0.50		psychiatric patients
HALME *et al.* 1957	83	1.01	2.46	0.18	0.56	"	operation, in whole material
HALME *et al.* 1957	41	1.1	2.97	0.20	0.71		operation, in men
HALME *et al.* 1957	36	0.90	1.62	0.15	0.41		operation, in women
ZUIDEMA *et al.* 1957	3	2.18	[illegible]	1.04	3.87		G tolerance, anxiety reaction
ZUIDEMA *et al.* 1957	3	3.89	11.9	0.89	3.03		G tolerance, anger reaction
v. EULER *et al.* 1959b	10	0.92	[illegible]	0.41	0.70		selected exciting movies
GOODALL & BERMAN 1960	9	1.5	1.6	0.8[illegible]	1.93	"	mock centrifugation
MENDELSON *et al.* 1960	10	1.03	2.09	0.28	0.7[illegible]	fluor	sensory deprivation
PEKKARINEN *et al.* 1961	189	0.75	0.81	0.22	0.5[illegible]	biol	matriculation examination
FRANKENHAEUSER & POST 1962	10	1.2	1.43	0.5	0.91	fluor	psychological tasks

served that if the excretion of noradrenaline exceeded 180 μg/24 hours, pheochromocytoma caused hypertension in 77 per cent of the cases (GOODALL & BOGDANOFF 1961). In 18 patients with a systolic blood pressure above 200 mmHg, and also in those with a systolic blood pressure of 150 to 200 mmHg, the noradrenaline excretion was clearly below the normal (MØLLER *et al* 1957). In hypertensives, the ratio of total and free adrenaline and noradrenaline normally 1.8, rises to 4.3, probably due to increased conjugation (KRONEBERG & SCHUMANN 1950). The noradrenaline and adrenaline content in the plasma is not raised in patients with essential hypertension in which neurogenic hypertension or pheochromocytoma have been excluded (MANGER *et al* 1954, GOLDFIEN *et al* 1961). Excretion of 3-methoxy-4-hydroxy mandelic acid in the urine is normal in patients with true essential hypertension (GITLOW *et al* 1960). In spite of no distinct changes in the amount of noradrenaline or adrenaline excreted in hypertensives having been observed, it is possible, however, that, in this disease, there is a sympathicotonic state which differs from the normal (v. EULER *et al* 1954b). This shows that the amount of noradrenaline and adrenaline excreted in hypertensives does not differ whether the patient is recumbent or upright (SUNDIN 1958).

Pheochromocytoma. Adrenal medullary tumours consist of cells with hormonal activity. Hence the excretion of noradrenaline or adrenaline or of both in the urine is often a hundred times increased (Table 7) and in exceptional cases as much as 600 times compared with the corresponding excretion in healthy people (ENGEL & v. EULER 1950, v. EULER 1951, LUND 1952, GOLDENBERG *et al* 1954, PEKKARINEN 1954, PEKKARINEN & PITKÄNEN 1955, v. EULER & FLODING 1956, PITKÄNEN 1956, v. EULER & STRÖM 1957, GITLOW *et al* 1960). In general, a correlation is observed between the noradrenaline and adrenaline content in the pheochromocytoma and the urinary excretion of these substances (ENGEL & v. EULER 1950). In the peripheral venous blood in which the normal adrenaline content is 0 to 1 μg/l and that of noradrenaline 0.2 to 1 μg/l (MANGER *et al* 1954, VALK & PRICE 1956, BENFEY & MELLAR 1957, PRICE & PRICE 1957, MANGER *et al* 1959), the concentration is often 20 times increased in patients with pheochromocytoma (LUND 1952, MANGER *et al* 1954, ROSS & TURNBULL 1955, MANGER *et al* 1959, SJOERDSMA *et al* 1959). In some cases the pheochromocytoma has been observed to contain an ab[illegible]

[illegible] of

et al 19[illegible]

activity

alone [illegible] mentioned substance may cause similar symptoms as do the latter two [illegible]

[illegible] the paragangliomas [illegible]

the normal, the SGOT changes parallelly, while the adrenaline content remains normal (Richardson *et al* 1960). Reserpine treatment does not prevent the increase in the urinary excretion of noradrenaline (Richardson *et al* 1960).

Table 6

Urinary excretion of noradrenaline and adrenaline in patients with cardiovascular diseases (µg/24 hours)

References	*n*	*Noradrenaline*	*Adrenaline*	*Method*	*Remarks*
Hypertension					
Burn 1953	7	80		*biol*	
v. Euler *et al* 1954 b	500	20—200	5—10		
Goldenberg *et al* 1954	35	7—100		" (hydrol.)	
Møller *et al* 1957	38	43		fluor	blood pressure *151—200* mmHg
Møller *et al* 1957	18	33		"	blood pressure > 200
Thomas 1957	37	8—163		" (hydrol.)	
Wilson 1960	14	5—45		*biol*	*women*
Wilson 1960	8	5—85		"	*men*
Cardiac infarction					
Forssman *et al* 1952	14	30—385	8.5—30	biol	
Forssman 1954	65		9.0	" (*hydrol.*)	
Cardiac insufficiency					
Pekkarinen et al 1960 a	94	18.5	7.0	biol	
Pekkarinen *et al* 1960 a	16	20.7	11.7	"	medium
Pekkarinen *et al* 1960 a	*9*	*20.5*	1.9		severe

Hypertension. It was first thought that essential hypertension is associated with increased excretion of urosympathin (Holtz *et al* 1947) but later, when the methods of investigation improved and larger series were available for study, it was considered that, in patients with essential hypertension the noradrenaline and adrenaline excretion is usually normal and rarely exceeds the normal (Goldenberg *et al* 1954, Pekkarinen & Pitkänen 1955, Hingerty 1957, Thomas 1957). In two series of 500 patients each the excretion of noradrenaline was considered normal in two thirds and distinctly increased in only about 15 per cent of the patients (v. Euler *et al* 1954b, Goodall & Bogdanoff 1961). It was further ob

served that, if the excretion of noradrenaline exceeded 160 μg/24 hours, pheochromocytoma caused hypertension in 77 per cent of the cases (GOODALL & BOGDANOFF 1961). In 18 patients with a systolic blood pressure above 200 mmHg and also in those with a systolic blood pressure of 150 to 200 mmHg, the noradrenaline excretion was clearly below the normal (MÖLLER *et al* 1957). In hypertensives, the ratio of total and free adrenaline and noradrenaline normally 1.8, rises to 4.3, probably due to increased conjugation (KRONEBERG & SCHUMANN 1950). The noradrenaline and adrenaline content in the plasma is not raised in patients with essential hypertension in which neurogenic hypertension or pheochromocytoma have been excluded (MANGER *et al* 1954, GOLDFIEN *et al* 1961). Excretion of 3 methoxy-4 hydroxy mandelic acid in the urine is normal in patients with true essential hypertension (GITLOW *et al* 1960). In spite of no distinct changes in the amount of noradrenaline or adrenaline excreted in hypertensives having been observed, it is possible, however, that, in this disease there is a sympathicotonic state which differs from the normal (v. EULER *et al* 1954b). This shows that the amount of noradrenaline and adrenaline excreted in hypertensives does not differ whether the patient is recumbent or upright (SUNDIN 1958).

Pheochromocytoma. Adrenal medullary tumours consist of cells with hormonal activity. Hence the excretion of noradrenaline or adrenaline or of both in the urine is often a hundred times increased (Table 7), and in exceptional cases as much as 600 times, compared with the corresponding excretion in healthy people (ENGEL & v. EULER 1950, v. EULER 1951, LUND 1952, GOLDENBERG *et al* 1954, PEKKARINEN 1954, PEKKARINEN & PITKÄNEN 1955, v. EULER & FLODING 1956, PITKÄNEN 1956, v. EULER & STRÖM 1957, GITLOW *et al* 1960). In general, a correlation is observed between the noradrenaline and adrenaline content in the pheochromocytoma and the urinary excretion of these substances (ENGEL & v. EULER 1950). In the peripheral venous blood in which the normal adrenaline content is 0 to 1 μg/l and that of noradrenaline 0.2 to 1 μg/l (MANGER *et al* 1954, VALK & PRICE 1956, BENFEY & MELVILLE 1957, PRICE & PRICE 1957, MANGER *et al* 1959), the concentration is often 20 times increased in patients with pheochromocytoma (LUND 1952, MANGER *et al* 1954, ROSS & TURNBULL 1955, MANGER *et al*, 1959, SJOERDSMA *et al* 1959). In some cases the pheochromocytoma has been observed to contain an abundance of dopamine which is excreted in the urine (v. EULER 1951, MANGER *et al* 1954, McMILLAN 1956, WEIL-MALHERBE 1956). Although the biological activity of dopamine is much weaker than that of noradrenaline and adrenaline, large amounts of the first mentioned substance may cause similar symptoms as do the latter two. The extramedullary tumours of the chromaffin cells, the paraganglionas, often contain only noradrenaline, and its ex-

Table 7

Urinary excretion of noradrenaline, adrenaline, and total catechol amines in patients with pheochromocytoma (µg/24 hours)

References	n	Noradrenaline Controls	Noradrenaline Pheochromocytoma	Adrenaline Controls	Adrenaline Pheochromocytoma	Total catechol amines Controls	Total catechol amines Pheochromocytoma	Method
Engel & v. Euler 1950	2		113—1240		15—780	20—40	200—1600	biol
v. Euler 1951	10	10—60	200—3000	2—10	15—600	12—70	215—3600	"
Lund 1952	4		420—3060		105—790	30—85	525—3150	fluor.
Burn 1953	3					16—86	180—3800	biol
Goldenberg *et al* 1954	16					7—100	190—2700	"
Pekkarinen 1954	1					31—185	430—2400	"
Davis *et al* 1955	1		720		160		880	
Knox & Slessor 1955	1		980—1780		450—1800			biol.
Pekkarinen & Pitkanen 1955	1					81	1354	fluor.
Ross & Turnbull 1955	1		1870		325			"
West & Taylor 1955						20—80	325—750	"
v. Euler & Floding 1956	10	2—177	187—14832	0—20	0—420	2—197	187—15252	fluor.
McMillan 1956	1		124—1054					chromat.
Pitkanen 1956	5	54	110—2400					fluor.
Subrahmanyam & Rao 1956	1		400—800			20—63	400—800	biol.
Weil-Malherbe & Heidelberg 1956	1	27—66	257	24—44	37		294	fluor.
v. Euler & Ström 1957	35		23—8800		0—780			"
Helmer & Sanders 1957	10					38—158	288—528	biol
Hingerty 1957	1						1500	fluor
Reutter *et al* 1957	1					40—100	100—175	"
Rosenberg 1957	1					250	2650	"
Thomas 1957	1					8—163	720	"
Benfey & Millar 1957	1	31	288	1	309	32	597	"
Healey & Mikelatos 1958	1	100	318	17	135			biol
Knight & Vacca 1958						40—100	472—1304	fluor.
de Graeff *et al* 1959	7		1300—2000					"
Sjoerdsma *et al* 1959	4						1000—2200	"
Bollman *et al* 1960	9						64—2780	"
Gitlow *et al* 1960	30	1 3[1]	163[1]	0 2[1]	17[1]			"
Haddy *et al* 1962	1		96 5[1]		3 5[1]			biol

[1] µg/100 mg creatinine

cretion in the urine may rise to 1354 μg/24 hours (PEKKARINEN 1954, PEKKARINEN & PITKÄNEN 1955) The urinary excretion of the most important metabolites of adrenaline and noradrenaline, 3 methoxy-4 hydroxy mandelic acid, which, in healthy persons, is on an average 4 mg/24 hours may rise ten times or more above the normal (SANDLER & RUTHVEN 1959, BOLLMAN *et al* 1960 GITLOW *et al* 1960, SUNDERMAN *et al* 1960, PISANO *et al* 1962)

In *postural hypotension*, the blood pressure falls rapidly in upright position due to the deficient regulating mechanism of the sympathetic nervous system (RAAB 1953) The amount of noradrenaline excreted in the urine of two patients confined to bed owing to postural hypotension was 2 7 to 13 0 μg/24 hours and that of adrenaline 0 6 to 2 8 μg/24 hours (LUFT & v EULER 1953)

In primary *Raynaud s disease* the adrenaline and noradrenaline content in the plasma is considered to be well over the normal limit noradrenaline 0 to 5 4 μg/100 ml adrenaline 0 to 4 4 μg/100 ml of blood on cooling the limb the adrenaline content increases to the double, and the noradrenaline content to four times the normal (PEACOCK 1959)

Renal diseases

In connection with renal insufficiency, pheochromocytoma may be suspected (MANGER *et al* 1954, KVALE *et al* 1957), since the noradrenaline and adrenaline content in the plasma is about ten times higher than normal as verified by the ethylenediamine method (WEIL-MALHERBE & BONE 1953) This is partly due to the plasma containing some unspecific substances since with the use of the trihydroxy indole method (LUND 1950), no changes are revealed in the plasma content of these substances in renal diseases (ZILELI *et al* 1958) The excretion of noradrenaline and adrenaline in the urine is sooner decreased in patients with renal insufficiency PEKKARINEN 1963)

Infectious diseases

No changes in the noradrenaline and adrenaline excretion *in infectious diseases* have as a rule been observed But, when inflammation of the central nervous system such as tuberculous meningitis, occurs, the excretion of both substances is comparatively great, the excretion of noradrenaline not exceeding 83 μg/24 hours and that of adrenaline not exceeding 22 μg 24 hours (DENGLER *et al* 1959) In test animals exposed to endotoxin shock the adrenaline content in the plasma increases to 20 μg/litre and the noradrenaline content to 15 μg/litre, and at the same time the

noradrenaline level changes in relation to the alterations in blood pressure (ROSENBERG *et al* 1961, SERAFIMOV 1962) Owing to the effect of anaphylactic shock, the plasma adrenaline probably increases slightly *and the nor*adrenaline content decreases, but the relationship is not as clear as in connection with hemorrhagic or endotoxin shock (MANGER *et al* 1957)

Mental diseases

In association with mental diseases the noradrenaline and adrenaline excretion often diverges from the normal (Table 8) Measured by the colorimetric method, the excretion of catechols in patients with *acute schizophrenia* increases from the normal 20 µg/24 hours, to 800 µg/24 hours, in some patients (SULKOWITCH *et al* 1957) Large amounts, especially of biologically active adrenaline, *18 to 36 µg/24 hours are excreted in acute* schizophrenics (GADDUM *et al* 1958, BERGSMAN 1959 CARLSSON *et al* 1959 a), but not in chronic schizophrenics (BERGSMAN 1959)

In *manic depressive mental disorders* the urinary excretion of adrenaline and noradrenaline is about twice as great in the manic period

Table 8

Urinary excretion of noradrenaline and adrenaline in psychiatric patients (µg/24 hours)

References	n	Noradrenaline		Adrenaline		Method	Remarks
Schizophrenia		*Controls*	*Patients*	*Controls*	*Patients*		
GADDUM *et al* 1958	10		34.4		30.1	fluor	
BERGSMAN 1959	18	31.8	40.8	10.1	11.9	biol (hydrol)	chronic
BERGSMAN 1959	6	31.8	59.2	10.1	18.2	biol (hydrol)	acute
CARLSSON *et al* 1959 a	11		33.3		5.0	fluor	
Manic depressive psychosis		Depressive	Manic	Depressive	Manic		
STRÖM OLSEN & WEIL MALHERBE 1958	12	27.9	93.9	9.7	19.5	fluor	
BERGSMAN 1959	16	31.8	55.7	10.1	30.5	biol (hydrol)	
CARLSSON *et al* 1959 b	19	45.1		24.1		fluor	
BERGSMAN 1959	8	31.8	47.0	10.1	6.9	biol (*hydrol*)	neurasthenia
BERGSMAN 1959	2	31.8	49.0	10.1	4.7	biol (hydrol)	mongolism
BERGSMAN 1959	12	31.8	50.0	10.1	4.9	biol (hydrol)	senile dementia

compared with that in the depressive period (Table 8), or in healthy persons (STROM OLSEN & WEIL-MALHERBE 1958, BERGSMAN 1959, CARLSSON et al 1959b)

In patients with *mongolism*, *neurasthenia*, or *senile dementia* the excretion of adrenaline is one half, and that of noradrenaline twice the normal (BERGSMAN 1959) The total catechol excretion in patients with psycho neurosis is twice to 20 times the normal (SULKOWITCH et al 1957)

Various other diseases

In patients with *duodenal ulcer* (Table 9) the excretion of noradrenaline is significantly less than in the controls especially by night, and the excretion of adrenaline is also slightly decreased (WADDELL et al 1960)

Unilateral *adrenalectomy does not affect the excretion of noradrenaline* or adrenaline in man (v EULER et al 1954a) but after bilateral adrenalectomy, the adrenaline excretion decreases significantly (Table 9), *often to less than 0.5 µg/24 hours but no distinct changes are observed in the excretion of noradrenaline* (HELLSTROM et al 1952, v EULER et al 1954a)

LOTILA & PEKKARINEN (1951) have *observed that, owing to the increased* activity of the adrenal gland, enlargement of the gland occurs and the adrenaline content falls by 40 per cent due to the effect of an

Table 9

Urinary excretion of noradrenaline and adrenaline in some pathological conditions (µg/24 hours)

References	n	Noradrenaline		Adrenaline		Method	Remarks
Thermal burn							
BIRKE et al 1957	9		125.0		48.0	biol	
GOODALL et al 1957	4		193.5		68.0	„	21–39 % burnt survived
GOODALL et al 19[illegible]	3		311.1		117.2	„	25–88 % burnt died
GOODALL et al 1957	3		172.6		133.8	„	31–50 % burnt, died
Adrenalectomy		Before	After	Before	After		
v EULER et al 1954a	15	41	27	5.4	5.1	biol	unilateral
v EULER et al 1954a	14	41	54	5.4	1.0	„	bilateral
Duodenal ulcer							
WADDELL et al 1960	10	57.1	31.4	12.0	7.7	fluor	

injurious factor of short duration. After traumatic death the content decreases to one third of the normal. Trauma, or *surgical operation with a normal course*, slightly augments the excretion of noradrenaline and adrenaline (Table 5) and a fivefold increase in the 17-OHCS content of the plasma occurs. But, if complications arise, the excretion of noradrenaline and adrenaline may be 3 to 15 times increased (FRANKSSON *et al.* 1954). During major thoracal or abdominal operations, HAME *et al.* (1957) and PEKKARINEN *et al.* (1957) observed that stimulation of the vegetative nervous system and of the pituitary-adrenocortical system increases the excretion of total 17-OHCS to 3 to 6 times the normal, and the excretion of 17-KS is only slightly raised. The average excretion of noradrenaline and adrenaline rises to twice the normal. In the present study the largest amount of noradrenaline excreted was 217.6 μg/24 hours and that of adrenaline 83.2 μg/24 hours. This was most distinctly evidenced on the first to third post-operative days. The excretion of vanilmandelic acid, the metabolites of noradrenaline and adrenaline, often increases after surgical operation to twice to three times the normal (PEKKARINEN *et al.* 1963).

Hemorrhagic shock due to profuse bleeding brings about rapid augmentation of the plasma level of adrenaline and noradrenaline (LUND 1951, MANGER *et al.* 1957, MILLAR *et al.* 1958). If the blood loss of a dog is 40 ml/kg in a short period, the adrenaline content increases to 12.5 μg/l or to 21 times the normal (WATTS & BRAGG 1957), at a later stage to 24.0 μg/l, before death due to loss of blood to 64 μg/l, on an average, and in exceptional cases to 220 μg/l (WALTON *et al.* 1959). In the same animals the noradrenaline content increases less, generally 1.5 to 20 times (MANGER *et al.* 1957, ROSENBERG *et al.* 1961), yet there is a distinct correlation to the amount of blood lost (ROSENBERG *et al.* 1961) — but some authors have observed no relationship between, on the one hand, the duration of the shock and the amount of blood lost, and, on the other, the increase in noradrenaline excretion (MANGER *et al.* 1957, GLAVIANO *et al.* 1960).

In severely *burnt* patients the urinary excretion of noradrenaline varies between 78 and 570 μg/24 hours and that of adrenaline between 25 and 260 μg (Table 9). The amount of the substances excreted depends on the degree of the burn. Yet in some severely burnt patients who did not survive, only small amounts of adrenaline were excreted, evidently due to insufficiency of the adrenals (GOODALL *et al.* 1957, BIRKE *et al.* 1957).

Noradrenaline and adrenaline metabolites *in the urine*

The adrenaline and the noradrenaline liberated from the adrenal medulla and the sympathetic nerve fibres is excreted, partly in the biologic

ally active form in the urine (HOLTZ *et al* 1947, v EULER & HELLNER 1951) but mainly as their metabolites

Intravenously infused noradrenaline is excreted in the urine in only 1 5 to 6 0 per cent (v EULER & LUFT 1951, GOLDENBERG 1951, PEKKARINEN & PITKÄNEN 1955, ELMADJIAN *et al* 1956 b) and that of adrenaline in smaller amounts than noradrenaline, 0 5 to 2 0 per cent (v EULER *et al* 1954 c, ELMADJIAN *et al* 1956 b, RESNICK & ELMADJIAN 1958) After subcutaneous injection of adrenaline and noradrenaline into man, 2 3 per cent of the former and 3 8 per cent of the latter is recovered in the biologically active free form (v EULER & ZETTERSTROM 1955)

Radioactive adrenaline infused into man is excreted unchanged in 1 to 5 per cent (RESNICK & ELMADJIAN 1958, GOODALL *et al* 1959) and that of noradrenaline in 4 per cent (GOODALL *et al* 1959)

In 1940, RICHTER after administering large doses of adrenaline recovered considerable amounts of the substance in the conjugated form In plasma, the conjugated adrenaline content increased 100 times compared with that of free adrenaline before administration (HÄGGENDAL 1963) Adrenaline has been observed to conjugate with sulphate (RICHTER 1940, BAYER & SCHAPIRO 1945) or with glucuronic acid (DODGSON *et al* 1947, CLARK & DRELL 1954) Almost all the adrenaline excreted in the urine was in the free form, but 60 per cent of the noradrenaline was combined with glucuronides (ELMADJIAN *et al* 1956 b)

On injection of radioactive noradrenaline into the blood stream, recovery in normal tissue is 10 times that in denervated tissue (HERTTING *et al* 1961 a) This observation suggests that part of the noradrenaline liberated from the nerve endings is re absorbed from the blood stream by the tissues Investigations performed later have revealed that the sympathetic nerve endings bind part of the noradrenaline liberated from the blood stream (HERTTING & AXELROD 1961)

Owing to the effect of O methyltransferase, the 3 methoxy derivatives normetanephrine and metanephrine are formed from noradrenaline and adrenaline The urine of rat contains both of these substances (AXELROD 1957 KRAUTT *et al* 1960) and also the urine of man (LABROSSE & MANN 1960) This observation was made even earlier on analysis of the urine of patients with phaeochromocytoma (LABROSSE *et al* 1958) Through oxidative deamination of monoamino-oxidase-enzymes, small amounts of 3 methoxy-4 hydroxyphenylglycol are formed from these metabolites and appear in the urine (AXELROD *et al* 1959), but 3 methoxy-4 hydroxymandelic acid is mainly formed (ARMSTRONG *et al* 1957) and thus is excreted in the urine of healthy persons in amounts of 0 7 to 11 0 mg/24 hours, patients with pheochromocytoma may excrete as much as 20 to 60 5 mg/24 hours (LABROSSE *et al* 1958 SANDLER & RUTHVEN 1959, SUNDERMAN *et al* 1960 PISANO *et al* 1962) In normal individuals the excretion of this substance

injurious factor of short duration. After traumatic death the content decreases to one third of the normal. Trauma or surgical operation with a normal course slightly augments the excretion of noradrenaline and adrenaline (Table 5) and a fivefold increase in the 17-OHCS content of the plasma occurs. But if complications arise the excretion of noradrenaline and adrenaline *may be 3 to 15 times increased* (FRANKSSON *et al.* 1954). During major thoracal or abdominal operations HAME *et al.* (1957) and PEKKARINEN *et al.* (1957) observed that stimulation of the vegetative nervous system and of the pituitary-adrenocortical system increases the excretion of total 17-OHCS to 3 to 6 times the normal, and the excretion of 17-KS is only slightly raised. The average excretion of noradrenaline and adrenaline rises to twice the normal. In the present study the largest amount of noradrenaline excreted was 217.6 μg/24 hours and that of adrenaline 83.2 μg/24 hours. This was most distinctly evidenced on the first to third postoperative days. The excretion of vanilmandelic acid, the metabolites of noradrenaline and adrenaline, often increases after surgical operation to twice to three times the normal (PEKKARINEN *et al.* 1963).

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follows the diurnal rhythm, the maximum being observed in the early afternoon (PEKKARINEN *et al* 1963)

The metabolite, 3,4 dihydroxymandelic acid, formed due to deamination of *noradrenaline and adrenaline, is also excreted in small amounts* in the urine (GOODALL *et al* 1958, v EULER *et al* 1959a, LABROSSE *et al* 1961)

EFFECT OF DRUGS ON THE URINARY EXCRETION OF NORADRENALINE AND ADRENALINE

Drugs acting on the central nervous system

Ethanol raises the urinary excretion of adrenaline in two hours to 9 to 12 times the normal (Table 10), and that of noradrenaline to 4 times the normal (ABELIN *et al* 1958) The excretion of adrenaline rises even with a 0 04 per cent blood content of ethanol although at this point there is still no change in the noradrenaline excretion (PERMAN 1958) In heavy drinkers, especially in those with delirium tremens the noradrenaline and adrenaline excretion is three times higher than normal (GIACOBINI *et al* 1960b) On administration of sublethal doses of alcohol to test animals, the excretion of adrenaline is 30 times augmented and that of noradrenaline 15 times (KLINGMAN & GOODALL 1957)

Table 10

Effect of centrally acting drugs on urinary excretion of noradrenaline and adrenaline (*µg/hour*)

References	n	Noradrenaline Before	Noradrenaline After	Adrenaline Before	Adrenaline After	Method	Remarks
Ethanol							
ABELIN *et al* 1958	12	1 50	[illegible]	0 53	4 65	[illegible]	
PERMAN 1958	43	0 81	1 04	0 29	0 48	fluor	
GIACOBINI *et al* 1960a	16	[illegible]	0 72	0 34	0 32	,	
GIACOBINI *et al* 1960b	17	0 76	[illegible]	[illegible]	0 58	,	alcohol intoxication
GIACOBINI *et al* 1960b	13	0 76	2 73	0 27	0 91		
Smoking							
WATTS & BRAGG 1956	11	1 90	1 79	0 19	0 61	fluor	

Anesthetics

Ethyl ether and *vinylether* have been observed to cause a fivefold increase in the plasma level of noradrenaline, both in man and test animals (RICHARDSON *et al* 1957

but, in man, decreases by 30 to 80

PRICE *et al*

per cent during the surgical phase of ether anesthesia and simultaneously, the blood pressure falls (KÄGI 1957). In connection with administration of *Pentothal*® and *Fluothane*® the blood pressure may decrease, because these drugs do not increase the adrenaline and noradrenaline content in the plasma (PRICE *et al* 1959, HAMELBERG *et al* 1960). Like ethyl ether, *Cyclopropane* which administered in normal doses does not decrease the blood pressure, increases the noradrenaline content to three times the normal; the adrenaline content remains unchanged (PRICE *et al* 1958, PRICE *et al* 1959).

Spinal anesthesia does not alter the noradrenaline and adrenaline content in plasma (HAMELBERG *et al* 1960).

Drugs stimulating the central nervous system

Pentamethylenetetrazol shock increases the adrenaline content in plasma by 130 per cent and that of noradrenaline by 40 per cent (WEIL-MALHERBE 1955a).

Lysergic acid diethylamide (LSD) increases the urinary excretion of noradrenaline and adrenaline 6 to 7 times in manic-depressive patients, but not in schizophrenics (ELMADJIAN *et al* 1957).

Analgetics

In test animals *morphine* decreases the adrenaline and noradrenaline content in the tissues (OUTSCHOORN 1952, VOGT 1954, GUNNE 1959, 1963, MAYNERT & KLINGMAN 1960). Morphine may increase the adrenaline secretion into the adrenal vein (STEWART & ROGOFF 1922, SATO & OHMI 1933, SATO *et al* 1935, WADA *et al* 1938) or the adrenaline content in the vena caval blood (PEKKARINEN 1948a). When the daily dose of morphine is gradually increased to a toxic level from 15 to 300 mg/kg, the adrenaline and noradrenaline excretion in rat increases very considerably, the excretion of adrenaline being still 13 times greater than normal, and that of noradrenaline significantly higher on the third day after termination of treatment; the noradrenaline excretion returns to normal in seven days (GUNNE 1961).

Drugs acting on the mediators of the sympathetic nervous system

Neuroleptic noradrenaline liberators

Reserpine. On first study of the effect of reserpine it appeared that, in rabbit and cat, the noradrenaline and adrenaline level in the heart, brain and adrenals decreases (BERTLER *et al* 1956, HOLZBAUER & VOGT

1956). Even with quite small doses, 0.005 mg/kg, a clear decrease in the noradrenaline content in the rabbit's heart is obtained in 16 hours (CARLSSON *et al.* 1957). With a dose of 0.015 mg/kg of reserpine the noradrenaline content decreases by 55 per cent in the rat's heart in six hours, and on increasing the dose to 0.1 mg/kg, the decrease is 89 per cent (PEKKARINEN *et al.* 1958). The noradrenaline level diminishes by 50 to 100 per cent in 20 minutes to 18 hours after administration of doses varying between 0.015 and 10 mg/kg, not only in the tissues of the heart, but also in other tissues, such as those of the intestines, liver and spleen, and of the sympathetic nervous system and ganglions, and, in general, wherever sympathetic nerve tissues are present (BRODIE *et al.* 1957, PAASONEN & KRAYER 1957, 1958, PEKKARINEN *et al.* 1958, DE SCHAEPDRYVER & PREZIOSI 1959). Five times larger doses of reserpine are required for equally rapid decrease of the noradrenaline level in the adrenals as in the tissues of the heart (CARLSSON *et al.* 1957).

It is well known that adrenaline (PEKKARINEN 1948) and noradrenaline (LUND 1950) injected into the blood circulation disappear rapidly, and radioactive adrenaline is adsorbed into the tissues (HERTTING *et al.* 1961 a). On injection of dopa into rabbits, dopamine is formed, and is found in the granular fractions of the brain, but after pre-treatment with reserpine, it appears in the soluble fraction (BERTLER 1961). Reserpine affects also the storage mechanism of noradrenaline by preventing entry and binding in the storage granules. Tests with radioactive noradrenaline (AXELROD *et al.* 1961, HERTTING *et al.* 1961 b) and adrenaline (WILSON *et al.* 1962) verify this view.

In samples taken from the adrenal vein of test animals, it has been observed that, in the beginning of reserpine treatment, large amounts of adrenaline and noradrenaline are liberated and enter the blood circulation (KRONEBERG & SCHUMANN 1958, STJÄRNE 1959, STJÄRNE & SCHAPIRO 1959). In the peripheral blood circulation of test animals the adrenaline, but not the noradrenaline, in the plasma increases after administration of reserpine (MUSCHOLL & VOGT 1958). Treatment for 18 to 24 hours with reserpine in doses of 1 to 4 mg/kg raises the urinary excretion of adrenaline in rat two to twentyfold, but that of noradrenaline is only doubled, simultaneously the adrenaline content in the adrenals decreases (DE JONGH 1958, HAZARD *et al.* 1960, BICKEL *et al.* 1961). In rabbit intravenous injection of 5 mg/kg decreases the urinary excretion of noradrenaline to almost nil, whereas the adrenaline excretion increases temporarily, but falls rapidly to normal again (CARLSSON *et al.* 1957).

In man, daily doses of 10 mg of reserpine (Serpasil®) bring about a decrease in the plasma noradrenaline level in three days from 6 to 2 μg/l, or to 33 per cent of the initial amount, but the adrenaline level remains

unchanged (Burger 1957). Correspondingly, reserpine decreases the excretion of noradrenaline (Table 11) in schizophrenics by 50 to 93 per cent (Gaddum *et al* 1958, Carlsson *et al* 1959a). After doses of 1 to 9 mg/day the excretion of adrenaline is the same as in the controls (Carlsson *et al* 1959a), or decreases even after one intramuscular injection of 15 mg (Gaddum *et al* 1958). When 0.2 to 0.9 mg of reserpine is given to pregnant women for 2 to 60 days before labour, there is no difference in the amount of noradrenaline and adrenaline excreted in the urine of the newborn infants of these mothers and that of the newborn of mothers not given reserpine (Castrén *et al* 1963). Some other rauwolfia alcaloids, such as raunescine decrease the noradrenaline content in brain and intestines (Kärki & Paasonen 1959).

Table 11

Drugs acting on the mediators of the sympathetic nervous system affecting the urinary excretion of noradrenaline and adrenaline (μg/24 hours)

References	n	Noradrenaline		Adrenaline		Method
Reserpine		Controls	Reserpine	Controls	Reserpine	
Gaddum *et al* 1958	10	41.4	15.4	32.0	14.4	*fluor*
Carlsson *et al* 1959a	11	33.3	4.5	25.0	26.6	"
MAO inhibitor		Controls	Marsilid	Controls	Marsilid	
Carlsson *et al* 1959b	8	37.5	46.5	30.6	17.6	*fluor*

Monoamine oxidase inhibitors

Iproniazide (Marsilid®), inhibits the enzymatic destruction of noradrenaline and adrenaline in tissues (Iisalo 1962), and given in doses of 50 mg/kg it raises the noradrenaline level in the tissues of rat by 36 to 79 per cent in 6 to 10 hours (Pekkarinen *et al* 1958). Daily doses of 10 to 15 mg increase the noradrenaline and adrenaline excretion in the urine of guinea pigs (Pekkarinen *et al* 1960b). On the other hand 100 mg/kg of malamide does *not affect the* urinary excretion of noradrenaline and adrenaline in rat (Bickel *et al* 1961). When iproniazide is given in doses of 20 mg/day to depressive patients (Table 11), the noradrenaline and adrenaline level is *not affected* (Carlsson *et al* 1959b).

Drugs inhibiting the sympathetic nervous fibres

Guanethidine blocks the postganglionic sympathetic nerve fibres. Doses of 25 to 150 mg cause no change however, in the noradrenaline and adrenaline level in the urine of *man* (*Iisalo* & Kärki 1963). In rabbit,

1956). Even with quite small doses, 0.005 mg/kg, a clear decrease in the *noradrenaline content* in the rabbit's heart is obtained in 16 hours (CARLSSON *et al.* 1957). With a dose of 0.015 mg/kg of reserpine the noradrenaline content decreases by 55 per cent in the rat's heart in six hours, and on increasing the dose to 0.1 mg/kg the decrease is 89 per cent (PEKKARINEN *et al.* 1958). The noradrenaline level diminishes by 50 to 100 per cent in 20 minutes to 18 hours after administration of doses varying between 0.015 and 10 mg/kg not only in the tissues of the heart *but also in other* tissues, such as those of the intestines, liver and spleen, and of the sympathetic nervous system and ganglions, and in general, wherever sympathetic nerve tissues are *present* (BRODIE *et al.* 1957, PAASONEN & KRAYER 1957, 1958, PEKKARINEN *et al.* 1958, DE SCHAEPDRYVER & PREZIOSI 1959). *Five times larger doses of reserpine are required for equally rapid decrease* of the noradrenaline level in the adrenals as in the tissues of the heart (CARLSSON *et al.* 1957).

It is well known that adrenaline (PEKKARINEN 1948) and noradrenaline (LUND 1950) injected into the blood circulation disappear rapidly, and radioactive adrenaline is adsorbed into the tissues (HERTTING *et al.* 1961 a). On injection of dopa into rabbits, dopamine is formed and is found in the granular fractions of the brain, but after pre-treatment with reserpine it appears in the soluble fraction (BERTLER 1961). Reserpine affects also the storage mechanism of noradrenaline by preventing entry and binding in the storage granules. Tests with radioactive noradrenaline (AXELROD *et al.* 1961, HERTTING *et al.* 1961b) and adrenaline (WILSON *et al.* 1962) verify this view.

In samples taken from the adrenal vein of test animals it has been observed that in the beginning of reserpine treatment large amounts of *adrenaline and noradrenaline are liberated and enter the blood circulation* (KRONEBERG & SCHÜMANN 1958, STJÄRNE 1959, STJÄRNE & SCHAPIRO 1959). In the peripheral *blood circulation of test animals the adrenaline* but not the noradrenaline in the plasma increases after administration of reserpine (MUSCHOLL & VOGT 1958). *Treatment for 18 to 24 hours with reserpine* in doses of 1 to 4 mg/kg raises the urinary excretion of adrenaline in rat two to twentyfold, but that of noradrenaline is *only doubled*, simultaneously the adrenaline content in the adrenals decreases (DE JONGH 1958, HAZARD *et al.* 1960, BICKEL *et al.* 1961). In rabbit intravenous injection of 5 mg/kg decreases the urinary excretion of noradrenaline to almost nil, whereas the adrenaline excretion increases temporarily but falls rapidly to normal again (CARLSSON *et al.* 1957).

In man daily doses of 10 mg of reserpine (Serpasil®) bring about *a decrease* in the plasma noradrenaline level in three days from 6 to 2 μg/l or to 33 per cent of the initial amount, but the adrenaline level remains

Table 12

Effect of certain drugs on urinary excretion of noradrenaline and adrenaline (µg/hour)

Reference	n	Noradrenaline Before	Noradrenaline After	Adrenaline Before	Adrenaline After	Method	Remarks
Insulin							
v. Euler & Luft 195[illegible]	10	2.40	1.56	0.23	3.30	biol (hydrol.)	
Elmadjian et al. 1956a	11	3.3	2.3	0.[illegible]6	[illegible]	biol (hydrol.)	
Luft & v. Euler 195[illegible]	20	1.1	1.5[illegible]	0.27	1.5[illegible]	biol	hypophysectomized patients
v. Euler et al. 1961	8			0.26	1.30	fluor	
v. Euler et al. 1961	8			0.1[illegible]	0.40	„	adrenalectomized patients
Metacholine							
Elmadjian et al. 1956a	10	4.0	4.9	0.33	0.41	biol (hydrol.)	
Zuidema et al. 195[illegible]	7	[illegible].53	3.79	1.0[illegible]	1.63	biol	
ACTH							
Luft & v. Euler 195[illegible]	11	1.91	0.68	0.[illegible]4	0.58	biol	[illegible]0 mg/day
Elmadjian et al. 1[illegible]	4	3.1	1.6	0.13	0.07	„ (hydrol.)	20 mg/day

Muscle relaxants

Apnea caused by infusion of *succinyl choline chloride* raises the blood pressure and the adrenaline content in plasma to 20 times the normal, and that of noradrenaline to 8 times the normal. When infusion of the drug is continued the blood pressure falls below the normal level but the adrenaline content in plasma rises more than 130 times and the noradrenaline content 13 times. When respiration starts again after infusion all the values mentioned rapidly return to normal (MILLAR 1960). This is probably mainly caused by the apneal state and not by the succinyl choline chloride since it has been observed that, if oxygenation is attended to, succinyl choline chloride decreases the adrenaline content to 60 per cent (WEIL-MALHERBE 1955b).

ENDOCRINE CHANGES IN TOXEMIC PREGNANCY

The development of toxemia of pregnancy is largely attributed to hypoxia of the placenta (YOUNG 1942) or of the uterine smooth muscle (MASTBOOM 1949). In consequence, hypoxia of the renal cortex may occur,

intravenous injection of 12.5 mg causes a decrease in the noradrenaline content in the heart as soon as within 4 hours, to about 15 per cent, but no change in this respect is noted in the brain and adrenals (CASS *et al.* 1960), 15 to 20 mg decrease the noradrenaline content by 2 to 45 per cent also in these organs, and in the cat brain by 45 to 52 per cent (SANAN & VOGT 1962). Also some other guanethidine derivatives decrease the noradrenaline content in tissues (KARKI *et al.* 1963).

Bretylium inhibits the release of noradrenaline from the sympathetic nerve fibres. It does not change or causes only a slight decrease in the noradrenaline content in the heart. Pre-treatment with bretylium clearly inhibits the decreasing action of reserpine on the noradrenaline and adrenaline level in the tissues (INESI *et al.* 1962, PEKKARINEN *et al.* 1962).

α-Methyldopa, which prevents transformation of dopa into dopamine, slightly decreases the excretion of vanilmandelic acid in the urine of patients with hypertension (SCHAUB *et al.* 1962).

Other drugs

Insulin, which causes hypoglycaemia, increases the adrenaline secretion from the adrenals (CANNON *et al.* 1924). After administration of insulin the adrenaline level in adrenal venous blood, often increases 20 times, or to 0.7 μg/kg/min (SATO *et al.* 1933, YEN *et al.* 1933) and according to chemical estimation, that in vena cava inferior rises to 2 to 8 μg/100 ml (PEKKARINEN 1948a). The highest adrenaline level in plasma is noted 30 to 45 minutes after administration of insulin (HOLZBAUER & VOGT 1954b, VENDSALU 1960). The adrenaline level in plasma decreases to almost half of the initial level, according to determination by the ethylenediamine method (WEIL-MALHERBE 1955a). In healthy people, or in test animals, the urinary excretion of adrenaline increases after administration of insulin to 5 to 23 times that in the controls (v. EULER & LUFT 1952, FRENCH *et al.* 1955, PITKÄNEN 1956, ELMADJIAN *et al.* 1956a). Insulin raises the adrenaline content to about the double also after adrenalectomy, and this may be caused by the reaction of the chromaffin tissues in other parts (v. EULER *et al.* 1961). The noradrenaline level in plasma does not change after administration of insulin (HOLZBAUER & VOGT 1954b, VENDSALU 1960), nor does the urinary excretion of this substance change (v. EULER & LUFT 1952, ELMADJIAN *et al.* 1956a, PITKÄNEN 1956).

ACTH and *cortisone* reduce the urinary excretion of noradrenaline (LUFT & v. EULER 1952). Since the amount of adrenaline excreted does not change, the decreased noradrenaline excretion must be due either to the increased production of adrenaline, or to the action of the mentioned substances on the sympathetic nerves, and not to inhibition of the renal excretion (LUFT & v. EULER 1952).

Table 12

Effect of certain drugs on urinary excretion of noradrenaline and adrenaline (µg/hour)

References	n	Noradrenaline Before	Noradrenaline After	Adrenaline Before	Adrenaline After	Method	Remarks
Insulin							
v. Euler & Luft 1952	10	2.40	1.56	0.23	3.30	biol. (hydrol.)	
Elmadjian *et al.* 1956a	11	3.3	2.3	0.36	1.64	biol. (hydrol.)	
Luft & v. Euler 1956	20	1.1	1.52	0.27	1.38	biol.	hypophysectomized patients
v. Euler *et al.* 1961	8			0.26	1.30	fluor.	
v. Euler *et al.* 1961	8			0.15	0.40	"	adrenalectomized patients
Methacholine							
Elmadjian *et al.* 1956a	10	4.0	4.9	0.33	0.41	biol. (hydrol.)	
Zuidema *et al.* 1957	7	2.57	3.72	1.02	1.63	biol.	
ACTH							
Luft & v. Euler 1952	11	1.91	0.68	0.74	0.58	biol.	50 mg/day
Elmadjian *et al.* 1956c	4	3.1	1.6	0.13	0.07	" (hydrol.)	20 mg/day

Muscle relaxants

Apnea caused by infusion of *succinyl choline chloride* raises the blood pressure and the adrenaline content in plasma to 20 times the normal, and that of noradrenaline to 8 times the normal. When infusion of the drug is continued the blood pressure falls below the normal level, but the adrenaline content in plasma rises more than 130 times and the noradrenaline content 13 times. When respiration starts again after infusion all the values mentioned rapidly return to normal (Millar 1960). This is probably mainly caused by the apneal state and not by the succinyl choline chloride, since it has been observed that, if oxygenation is attended to, succinyl choline chloride decreases the adrenaline content to 60 per cent (Weil-Malherbe 1955b).

ENDOCRINE CHANGES IN TOXEMIC PREGNANCY

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intravenous injection of 12.5 mg causes a decrease in the noradrenaline content in the heart as soon as within 4 hours to about 15 per cent but no change in this respect is noted in the brain and adrenals (Cass et al. 1960). 15 to 20 mg decrease the noradrenaline content by 2 to 45 per cent also in these organs and in the cat brain by 45 to 52 per cent (Sanan & Vogt 1962). Also some other guanethidine derivatives decrease the noradrenaline content in tissues (Kärki et al. 1963).

Bretylium inhibits the release of noradrenaline from the sympathetic nerve fibres. It does not change or causes only a slight decrease in the noradrenaline content in the heart. Pretreatment with bretylium clearly inhibits the decreasing action of reserpine on the noradrenaline and adrenaline level in the tissues (Inesi et al. 1962, Pekkarinen et al. 1962).

α-Methyldopa, which prevents transformation of dopa into dopamine, slightly decreases the excretion of vanilmandelic acid in the urine of patients with hypertension (Schaub et al. 1962).

Other drugs

Insulin, which causes hypoglycemia, increases the adrenaline secretion from the adrenals (Cannon et al. 1924). After administration of insulin the adrenaline level in adrenal venous blood often increases 20 times or to 0.7 µg/kg/min (Sato et al. 1933, Yen et al. 1933) and according to chemical estimation that in vena cava inferior rises to 2 to 8 µg/100 ml (Pitkänen 1948a). The highest adrenaline level in plasma is noted 30 to 45 minutes after administration of insulin (Holzbauer & Vogt 1954b, Vendsalu 1960). The adrenaline level in plasma decreases to almost half of the initial level according to determination by the ethylenediamine method (Weil-Malherbe 1955a). In healthy people or in test animals the urinary excretion of adrenaline increases after administration of insulin to 5 to 23 times that in the controls (v. Euler & Luft 1952, French et al. 1955, Pitkänen 1956, Elmadjian et al. 1956a). Insulin raises the adrenaline content to about the double also after adrenalectomy and this may be caused by the reaction of the chromaffin tissues in other parts (v. Euler et al. 1961). The noradrenaline level in plasma does not change after administration of insulin (Holzbauer & Vogt 1954b, Vendsalu 1960) nor does the urinary excretion of this substance change (v. Euler & Luft 1952, Elmadjian et al. 1956a, Pitkänen 1956).

ACTH and *cortisone* reduce the urinary excretion of noradrenaline (Luft & v. Euler 1952). Since the amount of adrenaline excreted does not change the decreased noradrenaline excretion must be due either to the increased production of adrenaline or to the action of the mentioned substances on the sympathetic nerves and not to inhibition of the renal excretion (Luft & v. Euler 1952).

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ELMADJIAN *et al.* 1956a	11	3.3	2.3	0.36	1.04	biol (hydrol.)	
LUFT & v. EULER 1956	20	1.1	1.5	[illegible]	1.58	biol	Hypophysectomized patients
v. EULER *et al.* 1961	8			0.96	1.30	fluor	
v. EULER *et al.* 1961	8			0.15	0.40	"	Adrenalectomized patients
Metacholin							
ELMADJIAN *et al.* 1956a	10	4.0	4.9	0.33	0.41	biol (hydrol.)	
ZUIDEMA *et al.* 1957	[illegible]	[illegible]53	[illegible]	1.09	1.6[illegible]	biol	
ACTH							
LUFT & v. EULER 1953	11	1.91	0.68	0.74	0.58	biol	[illegible] mg/day
ELMADJIAN *et al.* 1956e	4	3.1	1.6	0.13	0.07	(hydrol.)	[illegible]0 mg/hr

Muscle relaxants

Apnea caused by infusion of *succinyl choline chloride raises the blood* pressure and the adrenaline content in plasma to 20 times the *normal* and *that* of noradrenaline to 8 times the *normal*. When *infusion* of the drug is continued the blood *pressure falls below* the normal level but the adrenaline *content* in plasma *rises more than 130* times and the noradrenaline content 13 *times*. When *respiration starts* again after infusion *all the* values *mentioned* rapidly return to normal (MILLAR *1960*). This is probably mainly caused by the apneal state and *not by* the succinyl choline chloride since it has been *observed that, if oxygenation* is attended to succinyl choline chloride decreases *the adrenaline* content to 60 per cent (WEIL-MALHERBE 1955b).

ENDOCRINE CHANGES IN TOXEMIC PREGNANCY

The development of toxemia of pregnancy is *largely* attributed to hypoxia of the placenta (YOUNG 1942) *or of the* uterine smooth muscle (MASTBOOM 1949). In consequence hypoxia of the renal cortex may occur

resulting in edema, increased blood pressure and proteinuria (GOLDBLATT 1948), either reflectorily via the sympathetic nervous system (SOPHIAN 1953) or humorally via the blood circulation (PAGE & CORCORAN 1948)

In recent years, studies of the development of toxemic pregnancy have mainly been concerned with the hormone functions It has been observed that antidiuretic hormone, the product secreted by the posterior lobe of the hypophysis, normally occurs in measurable amounts in serum or plasma of pregnant women, but not in that of healthy persons (HAWKER 1957) Distinctly increased antidiuretic activity in serum has not been observed in toxemic pregnancy (LLOYD et al 1951, HAWKER 1957)

In early pregnancy, the urinary excretion of chorionic gonadotrophins is considerably raised but falls again in approximately the fourth month and remains constant up to term (LORAINE 1950 VENNING 1955) In toxemic pregnancy, no increase in the gonadotrophins in plasma, or the urinary excretion of these hormones has been regularly noted but in some patients the gonadotrophin level is exceptionally high (LORAINE & MATTHEW 1950, GOWAN 1951)

Progesterone is formed in the placenta (DICZFALUSY 1952, PEARLMAN 1957), likewise estrogens (MITCHELL & DAVIS 1954, DICZFALUSY & LINDKVIST 1956) During pregnancy the urinary excretion of pregnanediol the metabolite of progesterone is greatly increased (VENNING 1938 KLOPPER et al 1955, KANKAANRINTA & FORSSELL 1961), as is that of estrogens (BROWN 1956 DICZFALUSY & LAURITZEN 1961) The blood circulation in the placenta decreases in toxemic pregnancy (BROWNE & VEALL 1953), its function decreases, as does the urinary excretion of pregnanediol (BROWNE et al 1938, RUSSEL et al 1957 VENNING 1958 KANKAANRINTA 1963) and estrogens (SMITH & SMITH 1933 BROWNE et al 1938, VENNING 1958 TEN BERGE 1959, BORTH 1960) Thus the decreased formation and excretion of estrogens and progesterone is one of the most important endocrine changes observed during toxemic pregnancy

Adrenocortical function

When SELYE (1946) presented his theory that stress brings about diseased conditions in the organism these conditions were found to greatly resemble those occurring in toxemic pregnancy (PARVIAINEN et al 1950 b) The results of investigations performed show that the excretion of free corticoids in the urine slightly and variably increase during pregnancy (VENNING 1946 HEARD et al 1946 TOBIAN 1949 JAILER 1951 LLOYD et al 1952) or is normal (PFEIFFER et al 1952)

During toxemic pregnancy there is a slightly and variably increased excretion of free corticosteroids (TOBIAN 1949 PARVIAINEN *et al* 1950c) of free and conjugated formaldehydogenic steroids (VENNING *et al* 1954) and of total corticosteroids (LLOYD *et al* 1952)

The urinary excretion of total 17 OHCS (COHEN *et al* 1958, PEKKARINEN *et al* 1960c) and ketogenic 17 OHCS (APPLEBY & NORYMBERSKI 1957 LASTRAND 1958) is almost normal and that of 17 ketosteroids normal or slightly increased (VENNING 1946 BIRKE *et al* 1858) during normal and toxemic pregnancy ACTH tests have revealed that the urinary excretion of 17 ketosteroids is similar during normal and toxemic pregnancy (JAILER 1951) and the decrease in the number of eosinophils does not differ significantly from that in non pregnant women but the decrease is greater in the toxemic pregnant patients (SOIVA 1953)

During normal and toxemic pregnancy, the free 17 OHCS level in plasma is twice that in non pregnant women (GEMZELL 1953, ASSALI *et al* 1955 MIGEON *et al* 1957b PEKKARINEN *et al* 1962) In normal and toxemic pregnant patients the adrenocortical reserve is also similar or somewhat smaller than normal in the toxemic pregnant patients (GARST *et al* 1956) Intravenous infusion of ACTH further increases the plasma content of 17 OHCS during pregnancy (GARST & ASSALI 1956)

The concentration of 17 OHCS follows a diurnal rhythm (COHEN *et al* 1958 PEKKARINEN *et al* 1962) In late normal and toxemic pregnancy the free 17 OHCS level in plasma is twice the normal in the morning and three times higher in the evening than that in normal individuals whereas the conjugated 17 OHCS level remains unchanged throughout the 24 hours (PEKKARINEN *et al* 1962) No difference was observed between the average morning and evening values for free and conjugated 17 OHCS in plasma in a large series of pregnant women (PEKKARINEN *et al* 1962) The high concentration of free 17 OHCS in the plasma of pregnant women does not however signify that the renal function is impaired as no retention of conjugated 17 OHCS appears (PEKKARINEN *et al* 1962), like in patients with renal disease (KASANEN *et al* 1959)

The high plasma level of free 17 OHCS during pregnancy does not show clearly that the formation of hydrocortisone in the adrenals is increased This may be due to slow conjugation (PEKKARINEN *et al* 1962) or to retarded metabolization and decreased urinary excretion of 17 OHCS as the results of isotopic studies have shown (MIGEON *et al* 1957a)

But in corresponding investigations on the production rate of radioactive hydrocortisone it has been observed that formation of this substance is 2 to 2 5 times increased (COPE & BLACK 1959) Actually the adrenal cortex may be only slightly activated during pregnancy the retarded metabolism and the high plasma estrogen level explain why the free

17 OHCS level is high, and the retarded conjugation why the urinary excretion of conjugated total 17 OHCS is normal in spite of the high free 17 OHCS level in plasma

Adrenomedullary function

During intravenous infusion of very small doses of adrenaline and noradrenaline, the mediators secreted by the adrenal medulla and by the sympathetic nerve endings, the patient's blood pressure increases and the adrenaline causes metabolic changes in blood glucose, serum inorganic phosphorus, and blood lactic acid (HELVE & PEKKARINEN 1952), and proteinuria may occur on prolonged infusion of 15 to 44 μg/minute of noradrenaline and adrenaline (KING & BALDWIN 1955, 1956) Pretreatment with desoxycorticosterone (RAAB *et al* 1950) and with cortisone or ACTH (KURLAND & FREEDBERG 1951) sensitizes the blood vessels of persons given intravenous infusion of noradrenaline The blood pressure increase is greater after infusion of noradrenaline during toxemic pregnancy than during normal pregnancy (RAAB *et al* 1956), partly because the corticoid level in the plasma is high (PEKKARINEN *et al* 1962)

Table 13

Excretion of noradrenaline and adrenaline during normal and toxemic pregnancy
(μg/24 hours)

References	n	Noradrenaline		Adrenaline		Method
Pregnancy		Controls	Pregnancy	Controls	Pregnancy	
BURN 1953	7	59	56			Biol (hydrol.)
SUBRAHMANYAM 1959	25	22.8	36.2	5.7	6.5	Biol
Toxemia of pregnancy						
BURN 1953	6	59	58			Biol (hydrol.)
SUBRAHMANYAM 1959	107	33.8	68.[illegible]	5.7	10.4	Biol

BURN (1953), using a biological method, observed no increased urinary excretion of noradrenaline in seven women during late *normal pregnancy*, compared with that in non pregnant women (Table 13) RAAB & GIGEE (1954) *used the colorimetric method* by which all the catechols in the urine are determined, and noted that free catechols were excreted in amounts of 2.0 mg/24 hours by nine women in the sixth to ninth month of normal pregnancy, after hydrolysis 3.7 mg/24 hours, and by non pregnant women 2.2 mg/24 hours and 4.4 mg/24 hours, respectively SUBRAHMANYAM (1959)

determined biologically the free noradrenaline and adrenaline excreted in the urine of 25 women in late normal pregnancy the noradrenaline was determined on the cat's blood pressure and the adrenaline on the same cat's denervated nictating membrane or on the rat's uterus An average noradrenaline excretion of 36 2 µg/24 hours was noted in the pregnant women and 33 8 µg/24 hours in the controls. The excretion of adrenaline was 6 5 µg/24 hours in the former and 5 7 µg/24 hours in the latter In studies performed by the spectrophotometric method by which also dopamine was determined an increase in the catechol excretion was observed in late pregnancy (OESTERLING *et al* 1962)

At the beginning of *normal delivery* the urinary excretion of biologically active noradrenaline was 1 0 µg/hour and no significant changes were observed during delivery whereas the excretion of adrenaline at the beginning of delivery was 0 2 µg/hour increasing gradually to 0 5 µg hour (CENZELL *et al* 1956) Fluorimetric determination of the urinary excretion of both noradrenaline and adrenaline revealed no changes during spontaneous as well as during oxytocin induced delivery (BRUNDIN & ENGSTROM 1961)

In six women with *toxemic pregnancy* the total noradrenaline excretion was 57 µg/24 hours (BURN 1953) This amount did not differ from that excreted in non pregnant women or in healthy pregnant women in his material In 107 women with toxemia of pregnancy which included 100 cases of pre-eclampsia and seven of eclampsia the average blood pressure was 169 6/106 7 mmHg the average increase in weight during pregnancy was 10 kg more than that in the controls with normal pregnancy all the patients had proteinuria Their urinary excretion of noradrenaline was increased to 68 3 µg/24 hours on an average likewise the adrenaline excretion to 10 4 µg/24 hours or almost to twice the amount excreted in late normal pregnancy (SUBRAHMANYAM 1959)

The noradrenaline and adrenaline level in plasma during normal pregnancy (HOCHULI *et al* 1956 RIETZEL *et al* 1957 ISRAEL *et al* 1959 HOCHULI 1960 STONE *et al* 1960) or toxemic pregnancy (MANGER *et al* 1959 HOCHULI 1960) as well as during normal delivery (HOCHULI *et al* 1956 ISRAEL *et al* 1959) does not differ from that in non pregnant women If the uterine contractions during delivery are weak and painful a five to tenfold rise in plasma adrenaline occurs (HOCHULI *et al* 1956 RIETZEL *et al* 1957)

Treatment of toxemic pregnancy

Treatment of toxemic pregnancy in the present day is based on endeavours to increase the sodium chloride and water diuresis and to

17 OHCS level is high, and the retarded conjugation why the urinary excretion of conjugated total 17 OHCS is normal in spite of the high free 17 OHCS level in plasma

Adrenomedullary function

During intravenous infusion of very *small doses of adrenaline and nor adrenaline*, the mediators secreted by the adrenal medulla and by the sympathetic nerve endings the patient's blood pressure increases and the adrenaline causes metabolic changes in blood glucose serum inorganic *phosphorus and blood lactic acid* (HELVE & PEKKARINEN 1952), and proteinuria may occur on prolonged infusion of 15 to 44 μg/*minute of nor* adrenaline and adrenaline (KING & BALDWIN 1955, 1956) Pretreatment with desoxycorticosterone (RAAB *et al* 1950) and with cortisone or ACTH (KURLAND & FREEDBERG 1951) sensitizes the blood vessels of persons given intravenous infusion of *noradrenaline The blood pressure increase* is greater after infusion of noradrenaline during toxemic pregnancy than during normal pregnancy (RAAB *et al* 1956), partly because the corticoid level in the plasma is high (PEKKARINEN *et al* 1962)

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SUBRAHMANYAM 1959	25	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Toxemia of pregnancy						
BURN 1953	[illegible]	[illegible]	78			[illegible]
SUBRAHMANYAM 1959	[illegible]	[illegible]	[illegible]	[illegible]	10 4	Col

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PRESENT INVESTIGATION

PURPOSE OF STUDY

In the present investigation an endeavour was made to clarify the following points on the basis of the biologically active free noradrenaline and adrenaline excreted in the urine in late normal and toxemic pregnancy

1 Effect of normal and toxemic pregnancy as such on the activity of the sympathetic nervous system in the maternal organism
2 Excretion of noradrenaline and adrenaline during exertion
3 Effect of rest in bed on the excretion of noradrenaline and adrenaline
4 Diurnal variation in excretion of noradrenaline and adrenaline
5 Effect of reserpine treatment in toxemic pregnancy

METHOD

Preparation of samples for determination

In the present work v. EULER'S (1948 1956) method modified by PEKKARINEN (1962) was used for urinary determination of noradrenaline and adrenaline

For *adsorption* tests 25 ml of urine was taken, or 50 ml if the 24 hour output was large By means of a pH meter aided by an electrically driven stirrer system the pH of the samples was increased to 8 by adding 2 N NaOH and further to 8 5 by adding 0 5 N NaOH The sample was poured into a test tube into which 1 or 2 g of aluminium oxide (1 25), depending on the volume of urine had previously been measured (Al_2O_3 for chromatographic adsorption analysis according to Brockman, Merk A G Darmstadt) The test tubes were closed with a rubber stopper, and well shaken for 5 minutes to render adsorption more effective After shaking the samples were rapidly centrifuged and the Al_2O_3 rinsed twice in 25 to 50 ml of distilled water the pH of which was 6 5 to 7 0 The samples were centrifuged after each rinsing When six samples were treated at the same time about 20 minutes were required for adsorption

Elution was performed in 1 to 2 ml of 1 N H_2SO_4 depending on the volume of urine With the use of a methyl-orange indicator it was ascertained that the elution was acid enough the pH most often being about 2 The samples were frequently shaken in order to accelerate elution, centri

decrease the blood pressure by administration of certain hypotensive drugs. For patients with mild toxemia of pregnancy, whose main symptoms consist in edema and possibly in slightly increased blood pressure, some peroral saluretics are sufficient. In addition to decreasing the retention of excessive fluid and sodium (FRIEDBERG 1959, SALERNO *et al.* 1959) these saluretics reduce the increased blood pressure (FINNERTY *et al.* 1958). Yet development of toxemic pregnancy cannot be prevented by diuretics alone (WESLEY & DOUGLAS 1962).

Reserpine is the primary drug used for treatment of raised blood pressure during toxemic pregnancy, as even large doses of this drug do not decrease the blood pressure too much or too rapidly (HOCHULI 1961) and is thus suitable for use in the out-patients department. Good results have also been obtained in toxemia due to a chronic disease such as essential hypertension, as prophylactic reserpine treatment often prevents the liability to toxemia (LANDESMAN *et al.* 1957) or inhibits the development of severe toxemia of pregnancy (RAURAMO & SALMI 1959).

The phtalazine derivatives Apresolin® (ASSALI & SUYEMOTO 1952) and Nepresol® (LANZ & HOCHULI 1955) as well as protoveratrine (Puroverin®) (KASER 1953, RAURAMO 1955) may improve the renal blood flow and decrease the blood pressure. If reserpine is administered simultaneously with these drugs, the doses may be reduced to avoid side effects, yet the blood pressure reducing action is not decreased (FINNERTY & SITES 1955, HOCHULI 1961, TACCI 1961).

Ganglion blocking drugs cannot be used for treatment of toxemic pregnancy (KASER 1953, HAUSER & BAUMGARTNER 1957), nor can such which block the postganglionic sympathetic nerve fibres (BARTORELLI *et al.* 1960) be employed as they decrease the primarily small renal blood flow.

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In the present work v. EULER's (1948, 1956) method modified by HENKARINEN (1962) was used for urinary determination of noradrenaline and adrenaline

For *adsorption* tests 25 ml of urine was taken or 50 ml if the 24 hour output was large. By means of a pH meter aided by an electrically driven stirrer system the pH of the samples was increased to 8 by adding 2 N NaOH and further to 8.5 by adding 0.5 N NaOH. The sample was poured into a test tube into which 1 or 2 g of aluminium oxide (1 : 25) depending on the volume of urine had previously been measured (Al_2O_3 for chromatographic adsorption analysis according to Brockman Merk A G Darmstadt). The test tubes were closed with a rubber stopper and well shaken for 5 minutes to render adsorption more effective. After shaking the samples were rapidly centrifuged and the Al_2O_3 rinsed twice in 25 to 50 ml of distilled water the pH of which was 6.5 to 7.0. The samples were centrifuged after each rinsing. When six samples were treated at the same time about 20 minutes were required for adsorption

Elution was performed in 1 to 2 ml of 1 N H_2SO_4 depending on the volume of urine. With the use of a methyl orange indicator it was ascertained that the elution was acid enough the pH most often being about 2. The samples were frequently shaken in order to accelerate elution centri

decrease the blood pressure by administration of certain hypotensive drugs. For patients with mild toxemia of pregnancy, whose main symptoms consist *in edema and possibly in slightly increased blood pressure*, some peroral saluretics are sufficient. In addition to decreasing the retention of excessive fluid and sodium (FRIEDBERG 1959, SALERNO *et al.* 1959), these saluretics reduce the increased blood pressure (FINNERTY *et al.* 1958). Yet development of toxemic pregnancy cannot be prevented by diuretics alone (WESELEY & DOUGLAS 1962).

Reserpine is the primary drug used for treatment of raised blood pressure during toxemic pregnancy, as even large doses of this drug do not decrease the blood pressure too much or too rapidly (HOCHULI 1961), and is thus suitable for use in the out-patients department. Good results have also been obtained in toxemia due to a chronic disease, such as essential hypertension, as prophylactic reserpine treatment often prevents the liability to toxemia (LANDESMAN *et al.* 1957) or inhibits the development of severe toxemia of pregnancy (RAURAMO & SALMI 1959).

The phtalazine derivatives Apresolin® (ASSALI & SUYEMOTO 1952) and Nepresol® (LANZ & HOCHULI 1955) as well as protoveratrine (Puroverin®) (KASER 1953, RAURAMO 1955) may improve the renal blood flow and decrease the blood pressure. If reserpine is administered simultaneously with these drugs, the doses may be reduced to avoid side effects, yet the blood pressure reducing action is not decreased (FINNERTY & SITES 1955, HOCHULI 1961, TACCI 1961).

Ganglion blocking drugs cannot be used for treatment of toxemic pregnancy (KASER 1953, HAUSER & BAUMGARTNER 1957), nor can such which block the postganglionic sympathetic nerve fibres (BARTORELLI *et al.* 1960) be employed, as they decrease the primarily small renal blood flow.

PRESENT INVESTIGATION

PURPOSE OF STUDY

In the present investigation an endeavour was made to clarify the following points on the basis of the biologically active free noradrenaline and adrenaline excreted in the urine in late normal and toxemic pregnancy

1. Effect of normal and toxemic pregnancy as such on the activity of the sympathetic nervous system in the maternal organism
2. Excretion of noradrenaline and adrenaline during exertion
3. Effect of rest in bed on the excretion of noradrenaline and adrenaline
4. Diurnal variation in excretion of noradrenaline and adrenaline
5. Effect of reserpine treatment in toxemic pregnancy

METHOD

Preparation of samples for determination

In the present work v. EULER's (1948 1956) method modified by LEKKARINEN (1962) was used for urinary determination of noradrenaline and adrenaline

For *adsorption* tests 25 ml of urine was taken or 50 ml if the 24 hour output was large By means of a pH meter aided by an electrically driven stirrer system the pH of the samples was increased *to 8* by adding 2 N NaOH and further to 8 5 by adding 0 5 N NaOH The sample was poured into a test tube into which 1 or 2 g of aluminium oxide (1 2 g) de*pending on the* volume of urine had previously been measured (Al_2O_3 for chromatographic adsorption analysis according to Brockman Merk A G Darmstadt) The test tubes were closed with a rubber *stopper*, and well shaken *for* 5 minutes to render adsorption more effective *After shaking* the samples were rapidly centrifuged and the Al_2O_3 rinsed twice in 25 to 30 ml of distilled water the pH of which was 6 5 *to* 7 0 The samples were centrifuged *after each* rinsing When six samples were treated at the same time about 20 minutes were required for adsorption

Elution was performed in 1 to 2 ml of 1 N H_2SO_4 depending on the volume of urine With the use of a methyl orange *indicator* it was ascertained that the elution was acid enough the pH most often being about 2 The samples were frequently *shaken* in order to accelerate elution centri

fuged, and the elution poured into smaller test tubes. The sediment was rinsed in 0.7 ml of distilled water and the rinsing water added to the samples after centrifugation. The pH of the samples was increased to 3.5 by adding 0.5 N NaOH using a pH meter and a magnetic mixer and 4 ml of absolute ethanol to which was added 5 mg of ascorbic acid/100 ml of ethanol, and 4 ml of acetone so as to preserve the noradrenaline and adrenaline. The samples were kept overnight in the refrigerator.

When the samples had been centrifuged and poured into new test tubes, 2 N H_2SO_4 was added until the colour of the indicator was reddish. Ethanol and acetone were evaporated by means of an air stream in a horizontal tube system specially devised for serial evaporation (PEKKARINEN 1962). To obtain a large evaporation surface, the capillary adsorption tube was placed horizontally inside a test tube. Finally, by adding NaOH the pH of the samples increased to 3.5 and the volume was filled up with distilled water. When the initial volume was 25 ml of urine, the final volume was as a rule 2.5 ml, and when the initial volume was 50 ml, an endeavour was made to concentrate the volume to 3.0 ml.

Determination

The noradrenaline content was biologically determined by its action on the cat's blood pressure according to v. EULER's (1948) application of BARSOUM & GADDUM's (1935) method. The weight of the cats ranged from 1.9 to 4.5 kg. Chloralose to which was added 2 per cent ethanol (PEKKARINEN 1962) was used for anesthesia (60 mg per kg of body weight intravenously). The cat was given artifical respiration with an ideal starling respiration pump, the volume varying between 50 and 70 ml. To stabilize the reflex variation in the cat's blood pressure, 2 mg/kg of atropine was intracutaneously injected and 0.1 mg/kg of ergotamine and 8 mg/kg of cocaine intramuscularly as suggested by v. EULER (1958). Cocaine is known to sensitize the blood pressure reaction to noradrenaline as it prevents transfer of the injected noradrenaline from the blood circulation to the tissue (HERTTING *et al.* 1961 b). To prevent a possible action of histamine on the sample, antihistamine (Antihisan®) was given in doses of 3 mg/kg intramuscularly (KARKI 1956). By this means sensitization of the cat's blood pressure response was also increased and the determinations rendered reliable. The activity ratio of noradrenaline to adrenaline was 0.1 to 0.5. An amount of 0.5 ml of the sample was injected into the femoral vein and the venous cannula was rinsed with 1 ml of sodium chloride after each injection.

Adrenaline was biologically determined by its action on the hen's rectal cecum according to PEKKARINEN's (1962) modification of v. EULER's

(1948) method. From the distal part of the rectal cecum, which has been proved to be more sensitive than the thin proximal part, a piece of about 5 cm was removed and immersed in a bath of 25 ml of fresh Tyrode solution at 38°C (HOKFELT 1951) for incubation. An oxygen stream was directed into the bath.

Stock solution I		Stock solution II	
NaCl	100.0 g	$NaHCO_3$	50.0 g
KCl	2.5 g	$NaH_2PO_4 \cdot H_2O$	2.5 g
$CaCl_2$ (dry)	5.0 g	Dissolved in 1000 ml of distilled water	
$MgCl_2 \cdot 6H_2O$	5.0 g		
Dissolved in 1000 ml of distilled water			

Eighty ml of solution I and 40 ml of solution II were separately diluted in 1000 ml of distilled water and combined. To the Tyrode solution was added 1 g of glucose per litre and 1 mg of ascorbic acid per 100 ml (PEKKARINEN 1962) which stabilizes and greatly sensitizes relaxation of the hen's rectal cecum to adrenaline.

Before immersion in the bath, the cecum was emptied and adhesions which might prevent the movements of the gut were removed. An adequate oxygen stream was directed into the bath. Determination was not started until the gut, after careful balancing, was both sensitive and stabilized.

The samples under test, 0.05 to 0.2 ml, were neutralized with 0.1 to 0.2 ml of a solution of $NaHCO_3$-NaH_2PO_4 and injected at intervals of 2 to 5 minutes depending on the rapidity of the recovery of the gut. The activity ratio of adrenaline to noradrenaline varied between 10 and 40. Noradrenaline bitartrate (Winthrop) and adrenaline base (Dr. T. Schuchardt, München) were used as the comparative standard for determination of noradrenaline on the cat's blood pressure and of adrenaline on the hen's rectal cecum. The noradrenaline and adrenaline was diluted 1 : 1000 in HCl and preserved in pH 2.5 to 3.0 as a stock solution and stored in the refrigerator. The stock solution was diluted before injection into the cat's blood circulation to 10^{-8} and into the hen's rectal cecum to 10^{-6}. When the samples had been examined, the noradrenaline and adrenaline content was computed from the equation presented by v. EULER (1948).

$$x = \text{l-noradrenaline in } \mu g/25 \text{ ml of urine} = \frac{A - a}{Q - q}$$

$$y = \text{l-noradrenaline in } \mu g/25 \text{ ml of urine} = A - xQ$$

in which A = µg of l-noradrenaline equivalents per 25 ml of urine determined on the hen's rectal cecum

fuged, and the elution poured into smaller test tubes. The sediment was rinsed in 0.7 ml of distilled water and the rinsing water added to the samples after centrifugation. The pH of the samples was increased to 3.5 by adding 0.5 N-NaOH, using a pH meter and a magnetic mixer, and 4 ml of absolute ethanol to which was added 5 mg of ascorbic acid/100 ml of ethanol, and 4 ml of acetone, so as to preserve the noradrenaline and adrenaline. The samples were kept overnight in the refrigerator.

When the samples had been centrifuged and poured into new test tubes, 2N-H_2SO_4 was added until the colour of the indicator was reddish. Ethanol and acetone were evaporated by means of an air stream in a horizontal tube system specially devised for serial evaporation (PEKKARINEN 1962). To obtain a large evaporation surface, the capillary adsorption tube was placed horizontally inside a test tube. Finally, by adding NaOH, the pH of the samples increased to 3.5, and the volume was filled up with distilled water. When the initial volume was 25 ml of urine the final volume was as a rule 2.5 ml, and when the initial volume was 50 ml, an endeavour was made to concentrate the volume to 3.0 ml.

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Stock solution I		Stock solution II	
NaCl	800 0 g	$NaHCO_3$	50 0 g
KCl	2 5 g	NaH_2PO_4, H_2O	5 g
$CaCl_2$ (dry)	5 0 g	Dissolved in 1000 ml of distilled water	
$MgCl_2$, $6H_2O$	5 0 g		
Dissolved in 1000 ml of distilled water			

Eighty ml of solution I and 40 ml of solution II were separately diluted in 1000 ml of distilled water and combined To the Tyrode solution was added 1 g of glucose per litre and 1 mg of ascorbic acid per 100 ml (PEKKARINEN 1962) which stabilizes and greatly sensitizes relaxation of the hen s rectal cecum to adrenaline

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$$x - 1 \text{ noradrenaline in } \mu g/25 \text{ ml of urine} = \frac{A - a}{Q - q}$$

$y = 1 -$

a = l noradrenaline equivalents in μg/25 ml of urine determined on the cat's blood pressure
Q = the ratio of l adrenaline to l noradrenaline in the determinations of the hen's rectal cecum, and
q = the activity ratio of l adrenaline to l noradrenaline in the cat's blood pressure

Statistical treatment

For statistical analysis of the results the following formulae were used for calculation of the mean standard deviation and the standard error

(1)
$$\bar{x} = \frac{\Sigma x_i}{n}$$

(2)
$$SD = \sqrt{\frac{1}{n-1} \sum_{i=1} (x_i - \bar{x})^2}$$

from which may be derived a more practical formula

(2')
$$SD = \sqrt{\frac{1}{n-1}\left[\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}\right]}$$

(3)
$$SE = \frac{SD}{\sqrt{n}}$$

x_i is an individual observation, n the number of observations and $\bar{x}$ the mean of the observations

Student's test (Cfr LINDER 1957) was used for determination of the significance The mean was calculated by determining t as follows

(4)
$$t = \frac{x}{SE}$$

The significance of the difference between the means $\bar{x}'$ and $\bar{x}''$ was determined by calculating t from the formula

(5)
$$t = \frac{\bar{x}' - \bar{x}''}{s} \sqrt{\frac{n_x \; n_x}{n_x + n_x}} \text{ in which}$$

(6)
$$s^2 = \frac{\sum_{1}^{x} (x' - \bar{x}')^2 + \sum_{1}^{n_x} (x'' - \bar{x}'')^2}{n_x + n_x - 2}$$

n_x is the number of x' observations, and $n_{x'}$ the number of x'' observations. The t values obtained were compared with FISHER & YATES' (1953) statistical tables, and the probability p obtained. In the present investigation, the mean and the difference between two means was considered significant if 0.01 < p ≦ 0.05, and highly significant if 0.001 < p ≦ 0.01.

In some tests it was considered useful to determine the confidence limits. They naturally depend on the size of the mean of the dispersion. The required Student's t is obtained from the table as determined by the point of co-ordination of p and n.

Reliability tests

Recovery. To ascertain the reliability of the method used, the recovery of noradrenaline and adrenaline from two urine samples (A and B) was examined (Table 14). To these samples were added different amounts of noradrenaline and adrenaline. The samples were treated exactly as in the method described, starting with 25 ml of the urine volume. The amounts of noradrenaline, 0.41 μg and 0.95 μg, and of adrenaline, 0.20 μg and 0.18 μg initially present, were of the same order as in other investigations. To these samples were added 0.5, 1.0, 2.0 and 3.0 μg of noradrenaline, and, correspondingly, 0.2, 0.4, 0.6 and 1.0 μg of adrenaline. The determinations were made in duplicate.

Table 14

Recovery tests in samples of 25 ml of urine to which is added noradrenaline and adrenaline

Noradrenaline

Sample	NA in sample	NA added	NA measured	NA recovered	%
A 1	0.41 μg	0.5 μg	0.84 μg	0.43 μg	86
2	0.41	1.0	1.04	0.63	63
3	0.41	2.0	2.17	1.76	88
4	0.41	3.0	3.05	2.64	88
				Mean	81.3
				SD	12.2
				SE	± 6.1
B 1	0.95	0.5	1.30	0.35	70
2	0.95	1.0	1.82	0.87	87
3	0.95	2.0	2.54	1.59	80
4	0.95	3.0	3.10	2.15	72
				Mean	77.3
				SD	7.8
				SE	± 3.9

Table 14 (contin.)
Adrenaline

Sample	A in sample	A added	A measured	A recovered	%
A 1	0.20 μg	0.2 μg	0.35 μg	0.15 μg	75
2	0.20	0.4	0.54	0.34	85
3	0.20	0.6	0.63	0.43	72
4	0.20	1.0	0.90	0.70	70
				Mean	75.5
				SD	6.7
				SE	± 3.3
B 1	0.18	0.2	0.35	0.17	85
2	0.18	0.4	0.42	0.24	60
3	0.18	0.6	0.65	0.47	78
4	0.18	1.0	1.14	0.96	96
				Mean	79.8
				SD	15.1
				SE	± 7.6

Recovery of noradrenaline from sample A was 81.3 ± 6.1 (63—89), and from sample B 77.3 ± 3.9 (70—87) per cent. Recovery of adrenaline from sample A was 75.5 ± 3.3 (70—85), and from sample B 79.8 ± 7.6 (60—96) per cent. In the present study, the standard noradrenaline and adrenaline samples were often included, and in these, the recovery percentages agreed with those given above.

MATERIAL

Twenty-four hour urine samples were taken of 233 women in late pregnancy for determination of noradrenaline and adrenaline. Fifty-eight of these women were under treatment at the Maternity Department of the Central Hospital, University of Turku, or at the Maternity Hospital of the City of Turku. One hundred and seventy-five 24-hour samples were taken at the Maternity Welfare Centres of Turku. In 133 patients, pregnancy had proceeded normally up to the time of starting collection of samples, and in 100 toxemia of pregnancy had developed. 27 of these were given reserpine treatment. The degree of physical work performed by the pregnant women is seen from the following subdivision: 154 were occupied with housework, 25 were working away from home, and 54 were resting. Grouping of the patients with toxemia of pregnancy followed the rules set up by The American Committee on Maternity Welfare. Accordingly, toxemia of pregnancy was divided into two groups: pure toxemia of pregnancy, and 'superimposed' toxemia of pregnancy due to chronic diseases, such as essential hypertension or renal disorders. Pure toxemia of

pregnancy, which develops after the twenty fourth week of pregnancy, is subdivided into three groups pre eclampsia levis, pre-eclampsia gravis, and eclampsia To the group pre eclampsia levis belong the patients with persisting swelling or weight increase of over 500 g a week, blood pressure 145/95—160/100 mm Hg, and proteinuria up to 2 g in 24 hours To the group pre-eclampsia gravis belong patients with two or more of the following symptoms considerable swelling weight increase of 1 to 3 kg a week, constant blood pressure over 160/100 mm Hg and proteinuria at least 3 g/24 hours This group includes also all patients with even one of the following symptoms general swelling anasarca, constant blood pressure 180/115 mm Hg or over, and proteinuria of at least 5 g/24 hours Pre eclampsia gravis is frequently associated with subjective symptoms such as headache visual disturbances and gastrointestinal pain. Since the conception prevails that pure toxemia of pregnancy does not develop until the twenty fourth week the present material was selected to include women in the twenty fourth week of pregnancy, but more often such with a duration of thirty weeks Hence the material is considered to illustrate conditions in late pregnancy

The women attending the Maternity Welfare Centres, who were selected for this study were given advise regarding collection of urine samples, and containers for the purpose were supplied At the same time the usual routine procedure was followed an external examination was performed, the patient was weighed the blood pressure measured, and the urine analysed for proteinuria The urinary samples [illegible]
to morning and fetched by [illegible]
maintain the noradrenaline [illegible]
sulphuric acid was added beforehand into the containers If determinations were not performed immediately it was ascertained that the pH of the urine was about 3 and the samples were transferred to the refrigerator

The controls include five non pregnant women working in the laboratory by day Their average noradrenaline excretion was 23 8 ± 5 2 μg/24 hours and that of adrenaline 5 2 ± 0 9 μg/24 hours

RESULTS

URINARY EXCRETION OF NORADRENALINE AND ADRENALINE IN LATE PREGNANCY IN WOMEN OCCUPIED WITH HOUSEWORK

Normal pregnancy

1 *Material*

In the 105 women belonging to this series 54 of which were primiparae and 51 multiparae, no excessive increase in weight, or rise in blood pressure

was noted, and no proteinuria occurred during the period of collecting the urine samples. The majority of the women were benefiting by the maternity leave granted by law, and all were occupied with their usual housework. The women studied were given only certain iron and vitamin preparations in addition to having their usual food.

Table 15

Urinary excretion of noradrenaline and adrenaline in women occupied with housework in late normal pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	µg/24 hours	A %	NA µg/kg	A µg/kg
1	19	I	25	58.8	900	20.4	3.9	16	0.347	0.067
2	23	I	29	76.5	1880	43.2	2.7	6	0.565	0.035
5	22	I	30	60.1	1070	21.4	2.0	9	0.354	0.032
6	19	I	21	60.9	620	12.0			0.197	
7	35	III	23	64.6	1930	18.0	3.8	18	0.279	0.059
8	27	I	27	67.0	1230	25.0	3.1	11	0.374	0.046
9	26	IV	28	70.0	1830	28.0	3.7	12	0.400	0.053
10	40	VI	23	78.0	1680	20.1	3.8	16	0.258	0.049
12	19	I	37	75.5	1760	30.5	6.2	17	0.404	0.082
13	22	III	37	61.5	1680	21.0	4.7	18	0.342	0.077
14	23	II	37	66.5	655	14.0	1.4	9	0.210	0.021
16	21	II	40	73.5	1165	10.5	3.0	22	0.143	0.041
19	18	I	33	75.0	1280	13.7	1.5	10	0.183	0.020
20	25	I	40	66.0	1130	12.2	3.4	22	0.185	0.052
23	25	III	38	78.0	1820	11.0	3.3	25	0.141	0.042
25	27	III	30	85.0	740	21.0	3.2	13	0.247	0.038
27	20	I	35	80.0	1100	29.0	2.3	7	0.363	0.029
28	25	III	37	60.0	1285	32.0	3.1	9	0.534	0.052
36	23	II	36	60.0	1020	32.0	2.2	7	0.535	0.037
37	18	I	37	75.0	980	33.3	8.6	21	0.444	0.114
38	32	I	37	79.0	1960	20.8	5.3	20	0.264	0.067
39	24	II	36	66.0	2130	29.5	3.2	10	0.447	0.048
41	18	I	35	55.0	1340	17.2	3.6	17	0.314	0.065
44	22	I	38	56.0	930	15.8	2.1	12	0.282	0.038
45	19	I	37	93.0	2050	17.6	6.5	27	0.190	0.070
46	23	II	40	71.2	1370	16.4	4.0	20	0.230	0.056
48	19	I	40	65.0	700	11.0	2.1	16	0.168	0.032
51	19	I	29	73.9	1010	20.0			0.271	
52	26	II	38	65.9	990	13.2			0.200	
55	20	II	36	80.5	1000	25.5	3.2	11	0.320	0.040
56	38	III	33	72.0	810	19.4	2.5	11	0.270	0.035
57	20	I	36	80.0	960	21.0	1.2	6	0.263	0.015
58	24	II	37	70.0	1030	26.8	2.2	8	0.380	0.032
59	21	I	33	75.0	810	15.5	1.5	10	0.207	0.020

Table 15 (contin.)

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA μg/24 hours	A μg/24 hours	A %	NA μg/kg	A μg/kg
64	1	III	28	65 0	860	60 0	6	11	0 2 0	0 1 0
6	24	II	3	65 0	920	24 9	6 0	20	0 283	0 093
18	20	I	31	61 0	1130	21 0	10 1	32	0 345	0 168
		1	3	4 0	1460	16 3	5 3	25	0 23	0 08
4	20	I	36	24 0	1090	15 3	4 9	24	0 163	0 0
87	26	II	41	0	9 0	11 2	2 5	1	0 118	0 07
99	3	III	3	8 0	1890	32 [illegible]	[illegible]	16	0 418	0 081
109	2[illegible]	V	34	[illegible] 0	1350	18 5	9 0	3	[illegible]243	0 118
111	24	II	3	0	1 10	36 0	4 5	12	0 500	0 06
114	24	III	36	6 0	18 0	23 4	1	2	0 349	0 106
11	21	I	3	6[illegible] 5	25 0	31 5	12 6	28	0 474	0 190
1 0	24	I	38	64 0	1540	27 4	3 6	12	0 428	0 05[illegible]
1 9	2	I	3	61 8	840	17 2	4 5	21	0 280	0 0
13	3	I	24	68 6	1620		2			0 10
133	24	I	25	63 8	560	10 1			0 158	
13	28	III	37	100 6	840		3 8			0 036
138	27	II	5	60 8	15 0	15 1	3 0	17	0 248	0 045
139	22	II	28	6 6	1740	12 5	2 0	14	0 18	0 030
142	26	II	3	5[illegible] 3	960	1 3	6 3	26	0 0	0 111
140	2	I	40	7 0	480	13 0	3 0	19	0 194	0 045
145	30	III	40	9 7	2110	18 6	3 4	15	0 194	0 036
14	26	I	2	50 0	1260	19 0	9.5	33	0 380	0 190
151	20	I	3	103 5	1310	15 8	3 0	16	0 132	0 0 9
1	8	I		5[illegible] 4	1000		5 6			0 100
155	2	I	37	5	1 20	10 4	2.3	18	0 158	0 07
1 0	19	I	9	[illegible]9 7	1100		4.5			0 065
1[illegible]1	3	II	34	6 0	700	12 0	1 6	12	0 216	0 0 9
1[illegible]	7	I	39	69.5	840	13 0	3 4	21	0 187	0 049
1[illegible]9	19	I	4	0 1	2140	18 5	3 0	14	0 264	0 043
1		I	2	63	2000	16 0	2 5	14	0 251	0 039
1 3	2	I	27	63 0	1200	31 2	5 1	14	0 49	0 091
I 4	39	I	6	80 1	1140	27 5	6 9	20	0.342	0 086
I	26	I	39	68 5	1140	11 5	2 0	15	0 168	0 0 9
18	2	II	33	53	1480	12 4	3 6	23	0 208	0 061
183	33	II	37	0 5	10 0	21 0	3 0	12	0 298	0 043
	3	II	38	48 5	530	15 0	2 1	12	0 310	0 043
04	30	II	35	61 8	1500	21 [illegible]	2 9	12	0,353	0 047
07	2	II	36	58 0	980	14 0			0 240	
8 8	37	II	37	67 3	1000	12 0	3 6	23	0 1 8	0 0 4
10	22	II	40	5 1	60	1 0	3 8	18	0 225	0 0 0
11	2	I	38	6 0	2150	2 0	2 6	18	0 184	0 040
214	23	II	38	64 0	1080	18.5	3 5	18	0 289	0 055
15	30	II	39	57 0	2120	25	4 3	15	0 447	0 0 5
21[illegible]	19	I	6	3 0	1200	14 6	[illegible]	1[illegible]	0 200	0 034
219	32	I	40	58 0	2000	12 0	6	36	0 208	0 116
—0	2	III	38	6 0	1680	22 [illegible]	6 0	37	0 168	0 090

Table 15 (contin.)

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
221	26	I	38	63.0	1120		7.0			0.111
222	20	II	41	61.0	1100	21.5	2.2	9	0.352	0.036
223	33	III	38	92.0	1420	20.4			0.222	
224	21	I	39	73.0	1700	51.0			0.700	
225	25	IV	38	76.5	2100	14.0	3.8	21	0.184	0.050
226	21	I	40	72.0	1940	24.8	3.9	13	0.245	0.054
227	20	II	35	61.0	1060	22.0			0.360	
229	33	II	33	79.0	820	13.1	1.5	10	0.166	0.019
230	31	I	40	78.0	1400	28.0	7.5	21	0.360	0.096
231	18	I	38	74.2	1160	18.5	2.8	13	0.250	0.038
232	21	III	39	75.0	1640	13.0	15.0	54	0.174	0.200
233	26	II	38	76.0	1630	11.0	4.9	31	0.145	0.065
236	21	I	36	75.5	1500	14.9	4.8	14	0.198	0.064
240	34	V	30	68.0	1340	19.3	6.8	26	0.284	0.100
241	26	III	36	69.5	1760	12.7	1.8	12	0.183	0.026
267	25	I	39	65.3	1400	12.6	4.1	15	0.193	0.063
272	27	II	35	68.8	1800		13.6			0.198
294	19	I	34	66.0	650	13.4	3.8	22	0.203	0.058
296	18	I	36	74.0	1240	14.0	5.5	28	0.190	0.074
297	18	I	35	71.0	700	22.5	2.1	9	0.317	0.030
311	25	I	37	64.5	1680	16.2	3.1	16	0.250	0.048
325	23	I	39	66.0	980	13.7	3.8	22	0.208	0.057
331	18	I	38	74.2	1160	17.8	12.6	41	0.240	0.170
339	23	II	35	58.5	680	12.8	3.4	21	0.220	0.058
341	26	II	38	76.5	1230	36.1	6.3	15	0.470	0.083
Mean	25		35	69.6	1305	19.70	4.39	17.6	0.288	0.063
SD					451	7.00	2.67	8.7	0.100	0.039
SE					±44	±0.70	±0.27	±0.9	±0.010	±0.004
n 103						99	97			
Primiparae										
Mean	23		35	69.7	1300	19.32	4.55	18.1	0.271	0.061
SD					455	7.10	2.69	8.2	0.113	0.043
SE					±62	±1.01	±0.38	±1.20	±0.016	±0.006
n 54						50	50			
Multiparae										
Mean	27		36	69.4	1309	20.09	4.22	17.5	0.305	0.062
SD					450	6.80	2.77	9.5	0.084	0.042
SE					±65	±0.97	±0.40	±1.4	±0.012	±0.006
n 52						49	47			

2. *Results*

The 24 hour excretion of noradrenaline was determined in the urine samples of 99 women in late pregnancy (Table 15), and that of adrenaline in 97 samples. The average noradrenaline excretion was 19.7 ± 0.70

(10.1—60.0) μg/24 hours and calculated per kilogramme of body weight, 0.29 μg/24 hours, the average adrenaline excretion was 4.4 ± 0.27 (1.2—15.0) μg/24 hours or 0.06 μg/kg/24 hours. The adrenaline per cent in the total amount of noradrenaline and adrenaline was 17.6 in late pregnancy of women occupied with housework.

The average output of urine in women with normal pregnancy was fairly large, 1305 ± 44 ml/24 hours, varying between 480 and 2520 ml/24 hours. In ten women the output was over 2000 ml/24 hours, in 35 over 1500 ml/24 hours, and only in 28 less than 1000 ml/24 hours.

The average age in this group was 25 (18—40) years. Only 18 women were over 30. Counting from the day of the latest menstrual period, the duration of pregnancy was on an average 35 (21—41) weeks. In only 19, the duration of pregnancy at the time of collecting the samples was less than 32 weeks and in 86 of the 105 women belonging to this group the duration was 9 or 10 months. Although the body weight of these women average 69.6 kg, varied within considerable limits, 48.5 to 103.5 kg, only 11 were over over 80 kg and 13 less than 60 kg.

As the first pregnancy is considered to be the most strenuous one a study of whether the noradrenaline and adrenaline excretion differed in the primiparae and multiparae was studied. In 54 primiparae whose pregnancy covered about 35 weeks the average urinary excretion of noradrenaline was 19.3 ± 1.0 (10.1—43.2) μg/24 hours and that of adrenaline 4.6 ± 0.38 (1.2—12.6) μg/24 hours. The average excretion of noradrenaline per kilogramme of body weight was 0.27 μg/24 hours and that of adrenaline 0.06 μg/24 hours. The average adrenaline per cent was 18.1 in the primiparae.

The average age of the primiparae was 23 (18—39) years, weight 69.7 (50.0—103.5) kg and output of urine 1300 ± 62 ml/24 hours.

The multiparae at the 36th week of pregnancy, on an average excreted 20.1 ± 1.0 (10.5—60.0) μg/24 hours of noradrenaline on an average and 4.2 ± 0.40 (1.4—15.0) μg/24 hours of adrenaline or 17.3 per cent of the total. Calculated per kilogramme of body weight the noradrenaline excretion was 0.31 μg/24 hours and that of adrenaline 0.06 μg/24 hours. The adrenaline per cent was 17.5.

The average age of the multiparae was 26.5 (20—40) years, weight 69.4 (48.5—100.6) kg and urine volume 1309 ± 63 ml/24 hours.

On comparison of the results obtained in the two groups primiparae and multiparae good concurrence was observed in weight and urinary excretion. The multiparae were on an average 3.5 years older and the duration of pregnancy on an average one week longer. Neither of these facts are of importance however with regard to the noradrenaline and adrenaline excretion noted in the two groups.

No statistically significant difference in the urinary excretion of noradrenaline and adrenaline in primiparae and multiparae occurred. Thus the greater strain of pregnancy on the primiparae, and the possibly increased housework of the multiparae evidently caused no such injurious factors that might be reflected by increased excretion of adrenaline or noradrenaline. As no difference was observed in the results obtained in these two groups of a large statistically advantageous material, it was considered unnecessary to keep the groups apart in my further investigations.

In the controls which consisted of healthy women engaged in laboratory work, of the same age groups as the pregnant women, the value obtained for urinary excretion of noradrenaline was on an average 23.8 ± 5.2 µg/24 hours, and that for adrenaline 5.2 ± 0.9 µg/24 hours. Laboratory work is quite comparable with housework as to bodily strain. The noradrenaline and adrenaline excretion in late normal pregnancy did not differ significantly from that in the healthy non-pregnant women. Thus the strain of pregnancy is not of such a degree that increased excretion of noradrenaline and adrenaline might result.

Toxemic pregnancy

1. Material

The 24 hour urinary excretion of noradrenaline was studied in 38 women and that of adrenaline in 37. The toxemic symptoms in these women were still so mild that only three, Nos 24, 34 and 90, were referred to the group pre-eclampsia gravis and the remainder to the group pre-eclampsia levis. Pure toxemia of pregnancy affects primarily primiparae, hence the group contains as many as 25 primiparae and only 13 multiparae. All the women had left their wage-earning occupation and were doing only their own housework during the period of collecting samples. In addition to the usual iron therapy, one patient No 171 was given daily doses of 500 g of chlorothiazide to reduce severe edema, and two patients received daily doses of 3 g of ammonium chloride for the same purpose. One patient had had 0.3 mg of reserpine daily for 40 days owing to increased blood pressure, but the treatment was terminated 40 days before the samples were taken. In all patients except one No 24 the hemoglobin percentage was over 10 g which, in general, is considered the border value for anemia of pregnancy. In this patient the hemoglobin content was 9.4 per cent.

2. Results

The average urinary excretion of noradrenaline in women with toxemic pregnancy occupied with housework was 24.1 ± 2.22 (11.0—80.0) µg

Table 16

Urinary excretion of noradrenaline and adrenaline in women occupied with housework in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA μg/24 hours	A μg/24 hours	A %	NA μg/kg	A μg/kg
3	28	II	32	71.2	1900	53.2	3.0	5	0.748	0.042
11	25	I	40	81.0	1155	13.8	6.3	31	0.170	0.078
13	24	I	33	71.5	1220	24.5	4.3	15	0.343	0.060
17	25	I	33	61.0	1480	12.0	4.7	28	0.197	0.072
18	18	I	40	72.0	1430	24.0	5.3	18	0.334	0.074
21	30	I	38	79.0	1110	11.1	3.1	22	0.140	0.032
23	32	III	37	73.0	1300	27.5	2.5	8	0.377	0.034
24	25	I	36	82.0	1220	20.5	2.4	11	0.250	0.028
26	33	I	37	72.0	1370	12.7	4.3	25	0.176	0.060
31	21	I	36	68.0	1625	43.9	4.5	9	0.645	0.066
32	32	II	34	94.0	1850	80.0	1.7	2	0.852	0.018
33	26	I	35	61.0	845	19.4	4.0	17	0.318	0.065
34	29	II	33	93.5	1900	26.6	8.4	24	0.285	0.090
42	20	II	39	60.0	1420	34.2	2.5	7	0.570	0.042
43	22	II	38	71.0	1600	21.2	7.2	25	0.299	0.101
47	21	I	40	78.5	1330	22.0	3.2	13	0.280	0.042
53	27	III	37	69.0	1900	12.5	2.1	14	0.184	0.031
54	17	I	33	69.5	1270	20.5	1.5	7	0.295	0.022
99	34	III	28	64.2	2030	34.0	5.3	14	0.530	0.082
102	22	I	37	62.0	870	18.7	7.1	28	0.302	0.115
124	24	I	39	69.5	1920	22.4	5.0	28	0.322	0.072
146	27	I	38	67.0	470	14.1	1.6	10	0.210	0.024
148	27	II	40	75.6	2140	41.0	16.0	28	0.543	0.213
150	19	I	36	85.0	700	11.0	1.6	13	0.130	0.019
153	32	II	38	68.5	780	25.6	2.2	8	0.375	0.032
156	36	I	40	73.3	1470	16.0	1.5	9	0.219	0.021
162	29	I	33	95.1	740	17.6	2.1	11	0.185	0.022
171	24	I	31	72.3	920	22.0	3.2	13	0.304	0.044
180	18	II	30	77.0	1730	24.9	7.8	24	0.324	0.101
209	23	III	39	66.4	950	15.0	2.1	12	0.226	0.032
266	31	I	39	61.2	580	17.4	5.0	22	0.284	0.082
274	31	II	37	92.7	1900	43.0	10.8	22	0.464	0.126
299	24	I	37	76.0	850	17.7	2.5	12	0.233	0.033
300	19	I	38	73.0	1000	26.5	3.9	12	0.360	0.049
335	17	I	38	67.0	980	14.2	8.5	37	0.212	0.127
337	22	I	38	73.0	980	18.2	6.3	26	0.250	0.086
371	21	I	34	61.0	1000	19.4			0.318	
374	31	I	28	78.9	1200	17.5	4.4	20	0.222	0.056
Mean	25		36	73.3	1291	24.16	4.53	17.0	0.327	0.062
SD					441	13.69	3.04	8.5	0.166	0.043
SE					±143	±2.22	±0.50	±1.4	±0.027	±0.007
n 38						38	37			

No statistically significant difference in the urinary excretion of noradrenaline and adrenaline in primiparae and multiparae occurred Thus the greater strain of pregnancy on the primiparae and the possibly increased housework of the multiparae evidently caused no such injurious factors that might be reflected by increased excretion of adrenaline or noradrenaline As no difference was observed in the results obtained in these two groups of a large statistically advantageous material, it was considered unnecessary to keep the groups apart in my further investigations

In the controls which consisted of healthy women engaged in laboratory work, of the same age groups as the pregnant women, the value obtained for urinary excretion of noradrenaline was on an average 23 8 ± 5 2 µg/24 hours, and that for adrenaline 5 2 ± 0 9 µg/24 hours Laboratory work is quite comparable with housework as to bodily strain The noradrenaline and adrenaline excretion in late normal pregnancy did not differ significantly from that in the healthy non pregnant women Thus the strain of pregnancy is not of such a degree that increased excretion of noradrenaline and adrenaline might result

Toxemic pregnancy

1 Material

The 24 hour urinary excretion of noradrenaline was studied in 38 women and that of adrenaline in 37 The toxemic symptoms in these women were still so mild that only three, Nos 24, 34 and 90 were referred to the group pre eclampsia gravis and the remainder to the group pre eclampsia levis Pure toxemia of pregnancy affects primarily primiparae hence the group contains as many as 25 primiparae and only 13 multiparae All the women had left their wage earning occupation and were doing only their own housework during the period of collecting samples In addition to the usual non therapy one patient No 171 was given daily doses of 500 g of chlorothiazide to reduce severe edema and two patient received daily doses of 3 g of ammonium chloride for the same purpose One patient had had 0 3 mg of reserpine daily for 40 days owing to increased blood pressure but the treatment was terminated 40 days before the samples were taken In all patients except one No 24, the hemoglobin percentage was over 10 g which in general is considered the border value for anemia of pregnancy In this patient the hemoglobin content was 9 4 per cent

2 Results

The average urinary excretion of noradrenaline in women with toxemic pregnancy occupied with housework was 24 1 ± 2 22 (11 0—80 0) µg

Table 16

Urinary excretion of noradrenaline and adrenaline in women occupied with housework in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
3	28	II	32	71.2	1900	53.2	3.0	5	0.748	0.042
11	25	I	40	81.0	1155	13.8	6.3	31	0.170	0.078
15	24	I	33	71.5	1220	24.5	4.3	15	0.343	0.060
17	25	I	33	61.0	1480	12.0	4.7	28	0.197	0.072
18	18	I	40	72.0	1430	24.0	5.3	18	0.334	0.074
21	30	I	38	79.0	1110	11.1	3.1	22	0.140	0.032
22	32	III	37	73.0	1300	27.5	2.5	8	0.377	0.034
24	25	I	36	82.0	1220	20.5	2.4	11	0.250	0.028
26	33	I	37	72.0	1370	12.7	4.3	25	0.176	0.060
31	21	I	36	68.0	1625	43.9	4.5	9	0.645	0.066
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34	29	II	33	93.5	1900	26.6	8.4	24	0.285	0.090
42	20	II	39	60.0	1420	34.2	2.5	7	0.570	0.042
43	22	II	38	71.0	1000	21.2	7.2	25	0.299	0.101
47	21	I	40	78.5	1350	22.0	3.2	13	0.280	0.042
53	27	III	37	68.0	1900	12.5	2.1	14	0.184	0.031
54	17	I	33	69.5	1270	20.5	1.5	7	0.295	0.022
90	34	III	28	64.2	2030	34.0	5.3	14	0.530	0.082
102	22	I	37	62.0	870	18.7	7.1	28	0.302	0.115
126	24	I	39	69.5	1920	22.4	5.0	28	0.322	0.072
146	27	I	38	67.0	470	14.1	1.6	10	0.210	0.024
148	27	II	40	75.6	2140	41.0	16.0	28	0.543	0.213
150	19	I	36	85.0	700	11.0	1.6	13	0.130	0.019
153	32	II	38	68.5	780	25.6	2.2	8	0.375	0.032
156	36	I	40	73.3	1470	16.0	1.5	9	0.218	0.021
162	28	I	35	95.1	740	17.6	2.1	11	0.185	0.022
171	24	I	31	72.3	970	22.0	3.2	13	0.304	0.044
180	18	II	30	77.0	1730	24.9	7.8	24	0.324	0.101
209	23	III	39	66.4	950	15.0	2.1	12	0.226	0.032
266	31	I	39	61.2	580	17.4	5.0	22	0.284	0.082
274	31	II	37	92.7	1800	43.0	10.8	22	0.464	0.126
299	24	I	37	76.0	850	17.7	2.5	12	0.233	0.033
300	19	I	38	73.0	1000	26.5	3.9	12	0.360	0.049
315	17	I	38	67.0	980	14.2	8.5	37	0.212	0.127
337	22	I	38	73.0	980	18.2	6.3	26	0.250	0.086
371	21	I	34	61.0	1000	19.4			0.318	
374	31	I	38	78.9	1200	17.5	4.4	20	0.222	0.056
Mean	25		36	73.3	1291	24.10	4.53	17.0	0.327	0.062
SD					441	13.69	3.04	8.5	0.166	0.043
SE					±143	±2.22	±0.50	±1.4	±0.027	±0.007
n 38						38	37			

No statistically *significant difference in the urinary* excretion of noradrenaline and adrenaline in primiparae and multiparae occurred. Thus the greater strain of pregnancy on the *primiparae, and the possibly increased* housework of the multiparae evidently caused no such injurious factors that might be reflected by increased excretion of adrenaline or noradrenaline. As no difference was observed in the results obtained in these two groups of a large statistically advantageous material, it was considered unnecessary to keep the groups apart in my further investigations.

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Toxemic pregnancy

1. Material

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2. Results

The average urinary excretion of noradrenaline in women with toxemic pregnancy occupied *with housework* was 24.1 ± 2.22 (11.0—80.0) µg

Table 16

Urinary excretion of noradrenaline and adrenaline in women occupied with housework in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
3	28	II	32	71.2	1000	53.2	3.0	5	0.748	0.042
11	25	I	40	81.0	1155	13.8	6.3	31	0.170	0.078
15	24	I	23	71.5	1220	24.5	4.3	15	0.343	0.060
17	25	I	33	61.0	1480	12.0	4.7	28	0.197	0.072
18	18	I	40	72.0	1430	24.0	5.3	18	0.334	0.074
21	30	I	35	79.0	1110	11.1	3.1	22	0.140	0.052
22	32	III	37	73.0	1300	27.5	2.5	8	0.377	0.034
24	25	I	38	82.0	1220	20.5	2.4	11	0.250	0.028
26	33	I	37	72.0	1370	12.7	4.3	25	0.178	0.010
31	21	I	36	68.0	1025	43.9	4.5	9	0.645	0.066
32	22	II	34	94.0	1850	80.0	1.7	2	0.852	0.018
33	26	I	35	61.0	845	19.4	4.0	17	0.318	0.065
4	29	II	33	93.5	1900	26.6	8.4	24	0.285	0.090
42	20	II	39	60.0	1420	34.2	2.5	7	0.570	0.042
43	22	II	38	71.0	1600	21.2	7.2	25	0.299	0.101
47	21	I	40	78.5	1330	22.0	3.2	13	0.280	0.042
53	27	III	37	68.0	1900	12.5	2.1	14	0.184	0.031
54	17	I	33	69.5	1270	20.5	1.5	7	0.295	0.022
90	34	III	28	64.2	2070	34.0	5.3	14	0.530	0.082
102	22	I	37	62.0	870	18.7	7.1	28	0.302	0.115
126	24	I	39	69.5	1920	22.4	5.0	28	0.322	0.072
146	27	I	39	67.0	470	14.1	1.6	10	0.210	0.024
148	27	II	40	75.6	2140	41.0	16.0	28	0.543	0.213
150	19	I	36	85.0	700	11.0	1.6	13	0.130	0.019
153	32	II	38	68.5	780	25.6	2.2	8	0.375	0.032
156	36	I	40	73.3	1470	16.0	1.5	9	0.219	0.021
162	28	I	35	95.1	740	17.6	2.1	11	0.185	0.022
171	24	I	31	72.3	920	22.0	3.2	13	0.304	0.044
180	18	II	30	77.0	1730	24.9	7.8	24	0.324	0.101
209	23	III	39	66.4	950	15.0	2.1	12	0.226	0.032
266	31	I	39	61.2	580	17.4	5.0	22	0.254	0.082
274	31	II	37	92.7	1800	43.0	10.8	22	0.464	0.126
[illegible]9	24	I	37	76.0	850	17.7	2.5	12	0.233	0.033
[illegible]00	19	I	38	73.0	1000	26.5	3.9	12	0.360	0.049
37[illegible]	17	I	38	67.0	980	14.2	8.5	37	0.212	0.127
337	22	I	38	73.0	950	18.2	6.3	26	0.250	0.086
371	21	I	34	61.0	1000	19.4			0.318	
374	31	I	38	78.9	1200	17.5	4.4	20	0.222	0.056
Mean	25		36	73.3	1291	24.10	4.53	17.0	0.327	0.062
SD					441	13.69	3.04	8.5	0.166	0.043
SE					±143	±2.22	±0.50	±1.4	±0.027	±0.007
n 38						38	37			

No statistically significant difference in the urinary excretion of noradrenaline and adrenaline in primiparae and multiparae occurred. Thus the greater strain of pregnancy on the primiparae and the possibly increased housework of the multiparae evidently caused no such injurious factors that might be reflected by increased excretion of adrenaline or noradrenaline. As no difference was observed in the results obtained in these two groups of a large statistically advantageous material, it was considered unnecessary to keep the groups apart in my further investigations.

In the controls, which consisted of healthy women engaged in laboratory work of the same age groups as the pregnant women, the value obtained for urinary excretion of noradrenaline was on an average 23.8 ± 5.2 μg/24 hours and that for adrenaline 5.2 ± 0.9 μg/24 hours. Laboratory work is quite comparable with housework as to bodily strain. The noradrenaline and adrenaline excretion in late normal pregnancy did not differ significantly from that in the healthy non-pregnant women. Thus the strain of pregnancy is not of such a degree that increased excretion of noradrenaline and adrenaline might result.

Toxemic pregnancy

1. Material

The 24-hour urinary excretion of noradrenaline was studied in 38 women and that of adrenaline in 37. The toxemic symptoms in these women were still so mild that only three (Nos. 24, 34 and 90) were referred to the group pre-eclampsia gravis and the remainder to the group pre-eclampsia levis. Pure toxemia of pregnancy affects primarily primiparae; hence the group contains as many as 25 primiparae and only 13 multiparae. All the women had left their wage-earning occupation and were doing only their own housework during the period of collecting samples. In addition to the usual iron therapy one patient (No. 171) was given daily doses of 500 g of chlorothiazide to reduce severe edema, and two patients received daily doses of 3 g of ammonium chloride for the same purpose. One patient had had 0.3 mg of reserpine daily for 40 days owing to increased blood pressure, but the treatment was terminated 40 days before the samples were taken. In all patients except one (No. 24) the hemoglobin percentage was over 10 g, which in general is considered the border value for anemia of pregnancy. In this patient the hemoglobin content was 9.4 per cent.

2. Results

The average urinary excretion of noradrenaline in women with toxemic pregnancy occupied with housework was 24.1 ± 2.22 (11.0—80.0) μg

EFFECT OF WORK ON THE URINARY EXCRETION OF NORADRENALINE AND ADRENALINE IN LATE PREGNANCY

Normal pregnancy

I Material

Fifteen women were studied in order to clarify the effect of physical work on the urinary excretion of noradrenaline and adrenaline in late pregnancy During the period of collecting the urine samples, all the women were doing physical work, seven being shop assistants, six factory workers, and one a domestic help Their own housework still remained to be done at the end of the day which considerably prolonged their working hours

In this group eight women were primiparae and six multiparae

Table 17

Urinary excretion of noradrenaline and adrenaline in women doing physical work in late normal pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
4	23	I	22	60.1	785	31.5	4.0	11	0.523	0.067
30	19	I	28	62.5	1320	15.8	7.3	32	0.254	0.117
49	22	II	34	66.0	1340	15.0	5.3	26	0.227	0.080
70	20	I	38	73.5	1440	38.4	8.0	17	0.524	0.110
93	30	I	33	74.6	710	42.5	6.2	13	0.570	0.094
96	32	II	32	66.0	1020	17.2	4.5	21	0.260	0.068
134	36	I	31	73.0	1810	28.2	3.6	11	0.386	0.050
135	36	II	22	59.1	2000	20.0	4.2	17	0.339	0.071
149	20	II	28	68.7	1150	15.2	4.6	23	0.221	0.067
163	34	II	33	71.0	1420	29.8	2.5	8	0.420	0.035
175	22	I	35	73.0	1040	19.7	6.5	25	0.270	0.089
176	24	I	33	68.3	1160	23.6			0.346	
178	26	III	33	67.0	1890	15.8	3.0	16	0.235	0.045
275	25	I	36	61.2	920	19.8	4.2	19	0.320	0.069
373	18	I	38	71.5	1200	23.8	5.3	16	0.339	0.074
Mean	26		31	68.2	1348	24.01	4.90	18.2	0.350	0.073
SD					385	8.90	1.65	6.7	0.116	0.023
SE					±103	±2.39	±0.46	±1.8	±0.031	±0.006
n 15						15	14			

24 hours (Table 16). Calculated per kilogramme of body weight the excretion was 0.33 μg/24 hours. The average 24 hour excretion of adrenaline was 4.5 ± 0.50 (1.5—16.0) μg or 0.06 μg/kg/24 hours. The average amount of adrenaline in this group of toxemia was 170 per cent. In the three patients belonging to the group pre-eclampsia gravis the average value for noradrenaline excretion was 27.0 μg/24 hours and that for adrenaline 5.4 μg/24 hours. Both of these values are only somewhat higher than those for excretion of noradrenaline and adrenaline in the whole group of patients with toxemia. It seems that the degree of toxemia of pregnancy does not greatly influence the excretion of noradrenaline and adrenaline. The women whose weight exceeded 80 kg excreted on an average 30.3 μg/24 hours of noradrenaline and 4.8 μg/24 hours of adrenaline. The women whose weight was less than 65 kg excreted correspondingly 23.6 μg/24 hours and 4.8 μg/24 hours. Recalculation of the above amounts of noradrenaline per kilogramme of body weight gave almost equivalent values: 0.34 and 0.35 μg/kg/24 hours. The 13 women with a weight increase over 15 kg during pregnancy excreted 26.0 μg/24 hours of noradrenaline and 3.8 μg/24 hours of adrenaline.

The 24 hour output of urine was 1291 ± 143 ml.

The average age of the patients in this group was 25 (17—34) years, the duration of pregnancy 36 (28—40) weeks and the body weight 73.3 kg (60.0—95.1) kg.

Conclusions

On comparison of the urinary excretion of noradrenaline and adrenaline in late normal and toxemic pregnancy it was observed that in the latter case the excretion of noradrenaline 24.1 μg/24 hours was significantly greater than the corresponding excretion 19.7 μg/24 hours in the former. The difference between the average excretion of adrenaline 4.4 μg/24 hours in late normal pregnancy and 4.5 μg/24 hours in late toxemic pregnancy was not statistically significant. On further comparison of these figures with those for excretion in non pregnant women it was noted that in the latter the noradrenaline excretion 23.8 μg/24 hours and the adrenaline excretion 5.2 μg/24 hours did not differ statistically. The groups of normal and toxemic pregnancy were similar with regard to age and occupational strain.

Owing to retention of fluid the weight of the patients with toxemic pregnancy was naturally higher than that of the women with normal pregnancy.

EFFECT OF WORK ON THE URINARY EXCRETION OF NORADRENALINE AND ADRENALINE IN LATE PREGNANCY

Normal pregnancy

1 *Material*

Fifteen women were studied in order to clarify the effect of physical work on the urinary excretion of noradrenaline and adrenaline in late pregnancy. During the period of collecting the urine samples all the women were doing physical work, seven being shop assistants, six factory workers, and one a domestic help. Their own housework still remained to be done at the end of the day which considerably prolonged their working hours.

In this group eight women were primiparae and six multiparae.

Table 17

Urinary excretion of noradrenaline and adrenaline in women doing physical work in late normal pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urin volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
4	23	I	22	60.1	785	31.5	4.0	11	0.523	0.067
30	19	I	28	62.5	1350	15.8	7.3	32	[illegible]	0.117
49	22	II	34	66.0	1340	15.0	5.3	26	0.227	0.080
70	20	I	38	73.5	1440	38.4	8.0	17	0.524	0.110
93	30	I	33	74.6	710	42.5	6.2	13	0.570	0.084
96	32	II	32	66.0	1620	17.2	4.5	21	0.260	0.068
134	36	I	31	73.0	1810	28.2	3.6	11	0.386	0.050
135	36	II	22	59.1	2000	20.0	4.2	17	0.338	0.071
143	0	II	28	68.7	1150	15.2	4.6	23	0.221	0.067
163	34	II	33	71.0	1420	29.8	2.5	8	0.420	0.035
155	22	I	35	73.0	1040	19.7	6.5	25	0.270	0.089
171	24	I	33	68.3	1160	23.6			0.346	
178	26	III	33	67.0	1880	15.8	3.0	16	0.235	0.045
275	35	I	36	61.2	990	19.8	4.2	18	0.320	0.069
373	18	I	39	71.5	1900	28.8	5.3	16	0.[illegible]39	0.074
Mean	26		31	68.2	1348	24.01	4.90	18.2	0.350	0.073
SD					385	8.90	1.65	6.7	0.116	0.023
SE					±103	±2.38	±0.46	±1.8	±0.031	±0.006
n 15						15	14			

2. Results

The average urinary excretion of noradrenaline in women doing physical work was 24.0 ± 2.38 (15.0—42.5) μg/24 hours (Table 17), or 0.35 μg per kilogramme of body weight. The average adrenaline excretion was 4.9 ± 0.46 (2.5—8.0) μg/24 hours, or 0.07 μg/kg/24 hours, and the average adrenaline per cent 18.2.

The average age of the women included in this group was 26 (19—36) years, the duration of pregnancy 31 (22—38) weeks, and the body weight 68.2 kg, varying only between 60.1 and 74.6 kg. The output of urine was 1348 ± 103 ml/24 hours.

Toxemic pregnancy

1. Material

The adrenaline excretion was determined in the urine of 10 women and the noradrenaline excretion in 11, toward the end of toxemic pregnancy. Of all these women, three were employed in factories, three as shop assistants and five as domestic servants. As soon as the first symptoms of toxemic pregnancy were manifested, they were advised to leave their wage-earning work which required bodily exertion. Hence it is possible that the physical strain in this group was not quite comparable with that in the group of women with normal pregnancy doing physical work. Naturally, the toxemia was not severe in these cases. There were two patients with pre-eclampsia gravis: No. 60, duration of pregnancy 31 weeks, increase in weight 14 kg, blood pressure constantly 160/95 mmHg, and, No. 372, blood pressure 160/110 mmHg, proteinuria, and considerable swelling. The remaining nine women had pre-eclampsia levis: their blood pressure was 145/95, on an average, and the increase in body weight varied between 15 and 21 kg. Nine of the women of this group were primiparae and two were multiparae.

2. Results

The average urinary excretion of noradrenaline was 26.3 ± 3.36 (15.2—55.0) μg, or 0.36 ± 0.04 μg calculated per kilogramme of body weight (Table 18). The average urinary excretion of adrenaline was 5.9 ± 1.03 (2.1—12.8) μg/24 hours, or 0.08 ± 0.01 μg/kg/24 hours. Both values were considered normal.

In these patients with mild toxemia of pregnancy, the output of urine was exceptionally high: average 1630 ± 140 ml/24 hours.

The average age in this group was 25 (21—34) years, duration of pregnancy 33 (28—39) weeks, and body weight 72.5 (56.0—86.0) kg

Table 18

Urinary excretion of noradrenaline and adrenaline in women occupied with physical work in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
29	21	I	35	66.5	1780	25.0	2.1	8	0.376	0.032
35	26	I	32	56.0	1950	23.5	5.6	19	0.420	0.100
40	21	I	36	66.0	960	28.2	3.6	11	0.428	0.055
60	23	I	31	79.5	960	30.0	12.8	30	0.378	0.160
62	22	I	28	86.0	1970	55.0	7.6	12	0.640	0.089
76	28	I	31	77.5	1690	15.2	5.0	25	0.196	0.064
78	21	I	28	67.5	1560	23.0	3.1	12	0.341	0.046
143	29	IV	31	77.3	1810	17.4	5.2	23	0.225	0.067
166	29	II	33	75.8	2420	17.2	4.0	19	0.227	0.053
360	24	I	38	80.0	1030	20.2			0.253	
372	34	I	39	65.5	1750	34.4	9.5	22	0.522	0.145
Mean	25		33	72.5	1630	26.25	5.85	18.1	0.364	0.081
SD					466	11.13	3.20	7.3	0.136	0.044
SE					±140	±3.36	±1.03	±2.3	±0.041	±0.014
n 11						11	10			

Conclusions

The excretion of noradrenaline and adrenaline during normal and toxemic pregnancy was within normal ranges The average noradrenaline excretion in women occupied with physical work in late toxemic pregnancy, 26.3 µg/24 hours, was somewhat higher than that in late normal pregnancy, 24.0 µg/24 hours, but the difference was not significant ($0.05 < p$) In late normal pregnancy, the increased strain of work caused a significant rise in the noradrenaline excretion in women doing housework 19.7 µg/24 hours In late toxemic pregnancy, no rise occurred, however in women doing housework the excretion was 24.1 µg/24 hours

The average 24 hour excretion of adrenaline in women doing physical work in late toxemic pregnancy, 5.9 µg, was slightly higher than that in late normal pregnancy 4.9 µg/24 hours, but this difference was not either significant ($p > 0.05$) In late toxemic pregnancy, the adrenaline excretion in physical workers was 1.3 µg greater than in those occupied at home This increase was not significant, however ($p > 0.05$)

2 *Results*

The average urinary excretion of *noradrenaline in women doing physical* work was 24.0 ± 2.38 (15.0—42.5) μg/24 hours (Table 17) or 0.35 μg per kilogramme of body weight. The average *adrenaline excretion* was 4.9 ± 0.46 (2.5—8.0) μg/24 hours or 0.07 μg/kg/24 hours and the average adrenaline per cent 18.2.

The average age of the women included in this group was 26 (19—36) years, the duration of pregnancy 31 (22—38) weeks and the body weight 68.2 kg, varying only between 60.1 and 74.6 kg. The output of urine was 1348 ± 103 ml/24 hours.

Toxemic pregnancy

1 *Material*

The adrenaline excretion was determined in the urine of 10 women and the noradrenaline excretion in 11 toward the end of toxemic pregnancy. Of all these women three were employed in factories, three as shop assistants and five as domestic servants. As soon as the first symptoms of toxemic pregnancy were manifested they were advised to leave their wage earning work which required bodily exertion. *Hence it is possible that the* physical strain in this group was not quite comparable with that in the group of women with normal pregnancy doing physical work. Naturally the toxemia was not severe in these cases. There were two patients with pre-eclampsia gravis: No. 60, duration of pregnancy 31 weeks, increase in weight 14 kg, blood pressure constantly 160/95 mmHg and No. 372, blood pressure 160/110 mmHg, proteinuria and considerable *swelling*. The remaining nine women had pre-eclampsia levis: their blood pressure was *145/95* on an average and *the increase in body weight varied between* 15 and 21 kg. Nine of the women of this group were primiparae and two were *multiparae*.

2 *Results*

The average urinary excretion of noradrenaline was 26.3 ± 3.36 (15.2—55.0) μg or 0.36 ± 0.04 μg calculated per kilogramme of body *weight* (Table 18). The average *urinary excretion* of adrenaline was 5.9 ± 1.03 (2.1—12.8) μg/24 hours or 0.08 ± 0.01 μg/kg/24 hours. Both values were considered normal.

In these patients with *mild toxemia of pregnancy* the output of urine was exceptionally high, average 1630 ± 140 ml/24 hours.

The average age in this group was 25 (21—34) years, duration of pregnancy 33 (28—39) weeks and body weight 72.5 (56.0—86.0) kg

Table 18

Urinary excretion of noradrenaline and adrenaline in women occupied with physical work in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA μg/24 hours	A μg/24 hours	A %	NA μg/kg	A μg/kg
29	21	I	35	66.5	1780	25.0	2.1	8	0.376	0.032
35	26	I	32	56.0	1950	23.5	5.6	19	0.420	0.100
40	21	I	36	66.0	960	28.2	3.6	11	0.428	0.055
60	23	I	31	79.5	960	30.0	12.8	30	0.378	0.160
52	22	I	28	86.0	1970	55.0	7.0	12	0.640	0.099
76	23	I	31	77.5	1690	15.2	5.0	25	0.196	0.064
78	21	I	28	67.5	1560	23.0	3.1	12	0.341	0.046
143	29	IV	31	77.3	1810	17.4	5.2	23	0.225	0.067
166	29	II	33	75.8	2420	17.2	4.0	19	0.227	0.053
360	24	I	38	80.0	1050	20.2			0.253	
172	34	I	39	65.5	1750	34.4	9.5	22	0.522	0.145
Mean	25		33	72.5	1630	26.25	5.85	18.1	0.364	0.081
SD					466	11.13	3.20	7.3	0.136	0.044
SE					±140	±3.36	±1.03	±2.3	±0.041	±0.014
n 11						11	10			

Conclusions

The excretion of noradrenaline and adrenaline during normal and toxemic pregnancy was within normal ranges. The average noradrenaline excretion in women occupied with physical work in late toxemic pregnancy, 26.3 μg/24 hours, was somewhat higher than that in late normal pregnancy, 24.0 μg/24 hours but the difference was not significant ($0.05 < p$). In late normal pregnancy, the increased strain of work caused a significant rise in the noradrenaline excretion in women doing housework 19.7 μg/24 hours. In late toxemic pregnancy, no rise occurred, however in women doing housework the excretion was 24.1 μg/24 hours.

The average 24 hour excretion of adrenaline in women doing physical work in late toxemic pregnancy, 5.9 μg, was slightly higher than that in late normal pregnancy 4.9 μg/24 hours, but this difference was not either significant ($p > 0.05$). In late toxemic pregnancy, the adrenaline excretion in physical workers was 1.3 μg greater than in those occupied at home. This increase was not significant, however ($p > 0.05$).

The almost unchanged percentage of adrenaline in women doing physical work or occupied at home in late normal and toxemic pregnancy shows that the slight increase in excretion of noradrenaline and adrenaline due to strain of work affected the two substances in the same degree.

In the two women with toxemic pregnancy doing physical work who were referred to the group pre-eclampsia gravis the excretion of noradrenaline (30.0 and 34.4 µg/24 hours) and of adrenaline (12.8 and 9.5 µg/24 hours) was higher on an average, but owing to the small number of patients it cannot be established to what extent the degree of toxemic pregnancy affected the excretion of noradrenaline and adrenaline in these women.

EFFECT OF REST ON THE URINARY EXCRETION OF NORADRENALINE AND ADRENALINE IN LATE PREGNANCY

Normal pregnancy

1. *Material*

Fourteen women were studied to shed some light on the effect of rest on the 24-hour excretion of noradrenaline and adrenaline in late normal pregnancy. These women were all admitted to hospital because of premature rupture of the fetal membranes or because of prolonged pregnancy. The urine samples were generally collected during the first day in hospital. The patients were given the usual food and in addition only iron therapy.

2. *Results*

The average urinary excretion of noradrenaline in late normal pregnancy was 13.7 ± 1.18 (9.9—24.8) µg/24 hours in the patients confined to bed (Table 19). Calculated per kilogramme of body weight the excretion was 0.20 µg/24 hours. The average urinary excretion of adrenaline was 5.1 ± 1.00 (1.8—12.0) µg/24 hours or 0.07 µg/kg/24 hours. The adrenaline content was markedly high, 25.6 per cent, mainly due to the small amount of noradrenaline excreted by the women included in this group.

In only three patients the 24-hour excretion of adrenaline exceeded 10.0 µg. In one of these, No. 257, the adrenaline excretion was 12.0 µg/24 hours. She was admitted to hospital because of the calculated time of parturition being more than two weeks overdue and for that reason she was nervous. The second patient, No. 259, whose adrenaline excretion was 10.4 µg/24 hours, was a V-para. All her previous infants had died

soon after birth In the third patient, No 260, the adrenaline excretion was 10 8 µg/24 hours, she was extremely nervous and a heavy smoker

The average age in this group was 28 (20—40) years, weight 71 5 (47 0—100) kg, and duration of pregnancy 39 (34—43) weeks and 24 hour output of urine 1049 ± 120 ml

Table 13

Urinary excretion of noradrenaline and adrenaline in women resting in bed in late normal pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg
186	22	II	35	80 7	1090	16 3	4 1	20	0 202	0 051
193	20	II	34	47 0	700	10 1	2 7	21	0 215	0 057
212	21	I	39	67 7	560	18 2			0 270	
243	29	I	43	75 2	1100	10 6	3 1	23	0 140	0 041
254	24	I	38	70 0	1400	13 4	2 7	17	0 192	0 033
255	25	II	36	56 4	2100	13 5	5 9	30	0 240	0 104
257	29	III	42	76 0	1600	16 0	12 0	43	0 210	0 158
259	33	V	39	79 0	1150	12 7	10 4	45	0 161	0 130
260	40	III	38	55 0	1100	12 1	10 8	47	0 220	0 198
280	20	III	39	68 7	550	13 2	3 1	19	0 192	0 045
286	26	I	40	72 1	650	10 4	1 8	15	0 144	0 025
290	40	III	39	59 4	550	11 0	2 0	14	0 185	0 030
329	28	II	42	100 0	1250	24 8	5 7	19	0 248	0 057
381	32	V	39	89 9	950	9 9	2 5	20	0 110	0 028
Mean	28		39	71 5	1049	13 73	5 14	25 6	0 195	0 074
SD					449	4 42	3 60	11 9	0 049	0 054
SE					±120	±1.18	±1 00	±3 3	±0 011	±0 015
n 14							14	13		

Toxemic pregnancy

1 *Material*

Almost all the 24 patients admitted to hospital due to toxemic pregnancy had severe symptoms 13 were referred to the group pre eclampsia gravis, and 11 to that of pre eclampsia levis In the patients with pre eclampsia gravis the average systolic blood pressure was 171 5 mm Hg and the diastolic blood pressure 114 6 mm Hg, and, correspondingly, in the group of pre eclampsia levis, 152 7 mm Hg and 99 1 mm Hg The average blood pressure of the patients in the whole group was 163 1/107 5 mm Hg In

Table 20

Urinary excretion of noradrenaline and adrenaline in women resting in bed in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Degree of edema	Blood pressure mm/Hg	Protein urin g/24 hours	Urine volume cc	NA μg/24 hours	A μg/24 hours	A %	NA μg/kg	A μg/kg	
136	31	V	39	68.0	+++	160/100	1.0	960	23.0			0.339		g
158	22	I	38	70.0	++	175/105	1.2	480	12.2			0.175		g
170	22	I	38	77.9	+++	165/120	3.6	1200	16.8			0.216		g
198	33	I	38	100.8	+++	160/100	0.[illegible]	1200	17.3	4.1	19	0.172	0.032	l
201	19	I	36	58.8	+	135/95	5.0	1950	15.6	9.2	37	0.266	0.157	l
205	19	I	37	59.8	—	180/110	0.4	1400	16.8	9.2	36	0.281	0.154	l
235	17	I	28	54.0	+	160/100	+	1040	8.3	3.5	30	0.154	0.065	l
238	2[illegible]	I	40	74.0	++	145/95	+	700	10.5	2.0	16	0.142	0.027	l
242	20	I	39	68.7	++	160/100	1.0	800	10.2	3.0	23	0.148	0.044	g
245	28	I	34	74.3	+	200/130	0.7	1100	13.3	6.0	31	0.179	0.080	g
258	43	III	36	107.0	++	210/115	—	800	25.3	6.0	19	0.236	0.076	g
287	22	I	34	63.[illegible]	—	145/95	1.0	850	8.3	2.7	25	0.131	0.043	l
[illegible]01	25	I	36	66.0	+++	160/120	1.5	1100	7.0	4.0	37	0.106	0.061	g
[illegible]02	34	II	[illegible]6	98.5	+	155/95	0.5	1200	[illegible]0.5	[illegible].7	18	0.310	0.068	l
313	23	I	38	87.3	+	165/1[illegible]0	—	1000	24.0			0.275		g
317	[illegible]7	I	40	73.8	++	170/120	1.5	850	13.6			0.184		g
318	[illegible]7	I	[illegible]5	97.0	++	170/110	0.8	650	12.6	11.5	48	0.130	0.119	g
347	[illegible]8	III	39	67.9	+	160/110	0.5	1000	26.8	3.5	12	0.395	0.052	g
[illegible]52	28	II	37	70.2	++	145/95	0.4	500	14.5			0.207		l
[illegible]61	[illegible]1	I	38	81.0	+	160/105	—	730	17.2	4.2	20	0.212	0.052	l
[illegible]67	34	I	[illegible]9	72.0	++	160/110	1.5	1000	15.2	6.4	[illegible]0	0.211	0.089	g
375	18	I	40	59.0	++	160/120	1.8	600	9.0	2.9	24	0.153	0.019	g
380	[illegible]6	I	35	67.0	++	150/85	1.0	700	8.8			0.131		l
383	[illegible]0	V	39	75.0	+	145/105	1.0	1300	15.0	4.8	24	0.208	0.064	l
Mean	27		37	75.0		16[illegible].1/107.5	1.[illegible]	943	15.49	5.28	2[illegible].5	0.[illegible]07	0.070	
SD								330	6.80	2.64	9.50	0.076	0.037	
SI								±70	±1.[illegible]8	±0.[illegible]4	±2.0	±0.016	±0.009	
n 24							19		21	17				

g Pre-eclampsia gravis
l Pre-eclampsia levis

19 patients the average proteinuria was 1 3 g/24 hours Eighteen of these were primiparae and six multiparae

2 *Results*

In the patients resting in hospital due to toxemic pregnancy the average noradrenaline excretion was 15 5 ± 1 38 (7 0—30 5) μg/24 hours (Table 20) or calculated per kilogramme of body weight 0 21 ± 0 02 μg/24 hours. The average noradrenaline excretion in the patients with pre-eclampsia gravis was 16 1 μg/24 hours and that in the patients with pre-eclampsia levis 14 8 μg/24 hours

The adrenaline excretion in 17 patients belonging to this group was determined and the average obtained was 5 3 ± 0 64 (2 0—11 5) μg/24 hours or 0 07 ± 0 01 μg/kg/24 hours The average excretion in the patients with pre eclampsia gravis was 5 8 μg/24 hours and that of the patients with pre eclampsia levis 5 2 μg/24 hours

The amount of adrenaline excreted in the patients resting in hospital owing to toxemic pregnancy was 26 5 per cent

The average age in this group was 27 (17—43) years and the duration of pregnancy 37 (28—40) weeks The output of urine was 943 ± 70 ml which is less than in any of the other groups The average body weight 71 5 kg on the other hand was higher than in the other groups This was due to the fact that to this group belonged untreated patients with severe toxemia of pregnancy Owing to retention of fluid and sodium the output of urine was decreased and the weight increased

Effect of rest in hospital on the excretion of noradrenaline and adrenaline in late pregnancy

1 *Material*

The effect of the first four days of hospital treatment on the urinary excretion of noradrenaline and adrenaline was studied in seven women in late normal pregnancy and the noradrenaline excretion was determined in 11 and the adrenaline excretion in nine women in late toxemic pregnancy The women with normally progressing pregnancy were admitted to hospital owing to premature rupture of the fetal membranes or to prolonged pregnancy In the group of patients with toxemic pregnancy six had pre eclampsia gravis and five pre-eclampsia levis

During the period of collecting the 24 hour urine samples the patients were given the usual hospital food some iron preparations but no labour inducing or blood pressure reducing drugs

2 *Results*

Normal pregnancy

Hospital treatment covering several days in late normal pregnancy caused no significant decrease in the average excretion of noradrenaline (Table 21, Fig. 1). *On the first day in hospital, the average excretion* of noradrenaline was 11.9 µg/24 hours and on the fourth 10.7 µg/24 hours. The adrenaline *excretion increased gradually, but not significantly, during the first three days* in hospital, being 5.4 µg/24 hours on the first day and 10.4 µg/24 hours on the *third*. The heart rate remained unchanged 82/minute on each of the first four days.

Toxemic pregnancy

In late toxemic pregnancy, the urinary excretion of noradrenaline *rose gradually* during the stay in hospital, the average on the first day being 14.9 µg/24 hours, on the second day 15.5 µb/24 hours, on the *third 18.9 µg/24 hours,* and on the fourth 19.4 µg/24 hours (Table 21, Fig. 2). Owing to the comparatively large dispersion, the increase was not significant, however. The excretion of adrenaline decreased slightly during the period of rest, being 5.7 µg/24 hours on the first day and 4.9 µg/24 hours on the fourth.

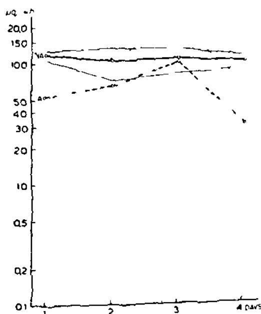


Figure 1

Average urinary excretion (with 95 % confidence limits) of noradrenaline (unbroken line) and *adrenaline* (broken line) in late normal pregnancy during the first four days in hospital. Semi logarithmic scale.

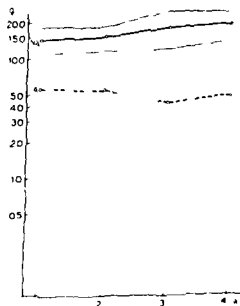


Figure 2

Average urinary excretion (with 95 % confidence limits) of noradrenalin (unbroken line) and adrenaline (broken line) during the first four days in hospital in patients with toxemia of late pregnancy. Semi logarithmic scale.

Table 21

Effect of rest in bed and simultaneous reserpine treatment on the urinary excretion of noradrenaline and adrenaline (with 95 % confidence limits) and on the toxemic symptoms in late pregnancy during the first four days in hospital

	Day	No	Noradrenaline µg/24 hours	Adrenaline µg/24 hours	Blood pressure mm Hg	Pulse rate /min	Urine volume cc	Proteinuria g/24 hours
Normal pregnancy	1	6	11.92 (10.99—12.85)	5.36 (2.56—8.16)		82		
	2	7	10.57 (7.36—13.78)	6.60 (2.09—11.11)		82		
	3	4	11.16 (8.57—13.75)	10.40 (0 —21.80)		82		
	4	3	10.70 (9.46—11.94)	3.54 (0.18—6.90)		82		
Toxemic pregnancy	1	10	14.85 (11.01—18.69)	5.67 (3.61—7.73)	173/115	81	955	0.8
	2	11	15.45 (11.94—18.96)	5.54 (3.87—7.21)	155/115	81	925	0.6
	3	7	18.85 (12.21—25.49)	4.36 (2.37—6.35)	164/105	77	1070	0.5
	4	6	19.40 (13.99—24.81)	4.89 (2.69—7.09)	143/102	78	1180	0.5
Toxemic pregnancy Reserpine treatment	1	10	15.10 (8.40—21.80)	5.37 (0 —12.15)	171/112	75	1260	1.6
	2	10	15.40 (12.42—18.38)	5.66 (4.13—7.19)	161/105	73	1270	1.2
	3	9	9.87 (5.99—13.57)	7.52 (3.86—11.18)	155/102	70	1290	1.3
	4	3	8.72 (0 —18.77)	8.95 (0 —42.75)	150/101	72	1310	1.5

The *symptoms of toxemia* of pregnancy were only slightly alleviated by the treatment in hospital (Table 21, Figs 3 and 4). The average blood pressure on the first day was 173/115 mm Hg, on the second 155/115 mm Hg, on the third 164/105 mm Hg, and on the fourth day 143/102 mm Hg. During the same period, the proteinuria decreased gradually from 0.8 g/24 hours to 0.5 g/24 hours. The increase in urine volume, from 955 ml/24 hours to 1180 ml/24 hours in four days, reveals the favourable effect of rest in hospital on toxemia of pregnancy. The average heart rate remained unchanged during the four days' treatment, being 81, 81, 77 and 78 beats/minute on the various days.

Conclusions

The average urinary excretion in the women resting in hospital in late normal pregnancy, 13.7 *µg/24 hours*, was highly significantly less than

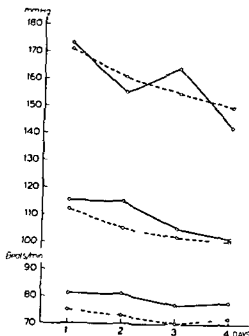


Figure 3
Effect of rest in hospital (unbroken line) and simultaneous reserpine treatment (broken line) on systolic (upper curve) and diastolic (middle curve) blood pressure, and pulse rate (lowest curve) in late toxemic pregnancy

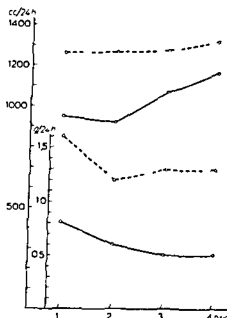


Figure 4
Effect of rest in hospital (unbroken line) and simultaneous reserpine treatment (broken line) on the output of urine (upper curves) and proteinuria (lower curves) in late toxemic pregnancy

the corresponding excretion in the women doing housework, 19.7 µg/24 hours, and in the physical workers, 24.0 µg/24 hours. Since, in addition, the average weight of the women resting in hospital during late normal pregnancy was higher than that of the women doing housework or physical work, the average noradrenaline excretion per kilogramme of body weight was naturally highly significantly less than in those doing housework or physical work.

In the patients resting in late toxemic pregnancy, the average noradrenaline excretion, 15.5 µg/24 hours, was somewhat greater than in the women with normal pregnancy, the difference was not significant ($p > 0.05$). But, in the women resting during late toxemic pregnancy, the excretion was highly significantly less, compared with that in the women doing housework, 24.1 µg/24 hours, and those doing physical work, 26.3 µg/24 hours.

Although the average noradrenaline excretion did not change in the first four days of treatment in hospital, being 11.9 µg/24 hours on the first day and 10.7 µg/24 hours on the fourth, the corresponding excretion in 11 patients with *toxemia of pregnancy increased during the same period*, from 14.9 µg/24 hours to 19.4 µg/24 hours. This increase was not signif-

icant however ($p > 0.05$) Since rest in hospital simultaneously reduced the toxemic symptoms decreased the blood pressure and proteinuria and increased the urine volume it seems as if there was no relationship between the symptoms of toxemia of pregnancy and the urinary excretion of noradrenaline as far as may be judged from this small material

The average urinary excretion of adrenaline in patients resting in hospital in late normal pregnancy 5 1 µg/24 hours or in late toxemic pregnancy 5 3 µg/24 hours did not differ significantly between the women with normal pregnancy doing housework 4 4 µg/24 hours or physical work 4 9 µg/24 hours and those with toxemia of pregnancy working at home 4 5 µg/24 hours or doing physical work 5 9/24 hours

EFFECT OF RESERPINE ON THE URINARY EXCRETION OF NORADRENALINE AND ADRENALINE IN LATE TOXEMIC PREGNANCY

Excretion in patients treated with reserpine

1 *Material*

The urinary noradrenaline and adrenaline excretion was determined in 27 patients with toxemia of pregnancy treated with reserpine Fifteen of these patients were primiparae and 12 multiparae Fourteen of the women treated with reserpine were in hospital ten at home benefiting by maternity leave and three were occupied with physical work When the treatment was started the patients were divided according to the degree of toxemia the group pre eclampsia gravis included 14 of which 12 were in hospital and two at home the group pre-eclampsia levis included 13 two of which were in hospital and eight at home three were still employed in physical work

The average dose of reserpine given was 0 5 (0 3—2 0) mg/24 hours In general the drug was administered by mouth except in a few cases when the patients with severe toxemia were given intramuscular injections immediately on admission to hospital The duration of treatment before starting the collection of samples was 21 2 (3—80) days on an average

2 *Results*

The average 24 hour excretion of noradrenaline determined in 27 women with toxemia of pregnancy treated with reserpine was 11 8 ± 1 20 (5 2—38 5) µg/24 hours (Table 22) In the women given reserpine who were doing physical work or housework the noradrenaline excretion was

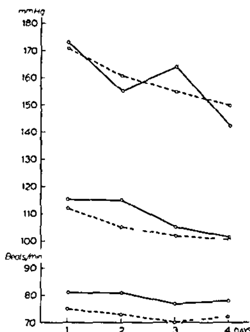


Figure 3
Effect of rest in hospital (unbroken line) and simultaneous reserpine treatment (broken line) on systolic (upper curve) and diastolic (middle curve) blood pressure, and pulse rate (lowest curve) in late toxemic pregnancy

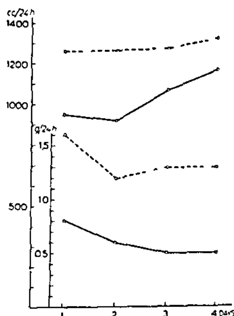


Figure 4
Effect of rest in hospital (unbroken line) and simultaneous reserpine treatment (broken line) on the output of urine (upper curves) and proteinuria (lower curves) in late toxemic pregnancy

the corresponding excretion in the women doing housework, 19.7 μg/24 hours, and in the physical workers, 24.0 μg/24 hours. Since, in addition the average weight of the women resting in hospital during late normal pregnancy was higher than that of the women doing housework or physical work, the average noradrenaline excretion per kilogramme of body weight was naturally highly significantly less than in those doing housework or physical work.

In the patients resting in late toxemic pregnancy, the average noradrenaline excretion, 15.5 μg/24 hours, was somewhat greater than in the women with normal pregnancy: the difference was not significant ($p > 0.05$). But, in the women resting during late toxemic pregnancy the excretion was highly significantly less, compared with that in the women doing housework, 24.1 μg/24 hours, and those doing physical work 26.3 μg/24 hours.

Although the average noradrenaline excretion did not change in the first four days of treatment in hospital, being 11.9 μg/24 hours on the first day and 10.7 μg/24 hours on the fourth, the corresponding excretion in 11 patients with toxemia of pregnancy increased during the same period from 14.9 μg/24 hours to 19.4 μg/24 hours. This increase was not signif

symptoms were observed which might have been thought to be caused by the low noradrenaline excretion

The average adrenaline excretion in the patients with toxemic pregnancy *given reserpine treatment was* 4.2 ± 0.44 (1.3—13.0) µg/24 hours. The amount of adrenaline excreted was 25.1 per cent

Calculated per kilogramme of body weight, the 24 hour excretion of noradrenaline was 0.17 µg and that of adrenaline 0.06 µg

The average 24 hour output of urine by the patients in this group was rather small, 1218 ± 80 ml. The average age was 27 (17—43) years and the duration of pregnancy 37 (31—41) weeks

Effect of reserpine on the excretion of noradrenaline and adrenaline in the first four days of treatment in hospital

1 *Material*

As it was observed that the noradrenaline excretion in patients treated with reserpine in late toxemic pregnancy was less than in the patients with toxemia and not given reserpine an attempt was made to

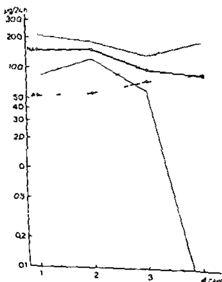


Figure 5
Average urinary excretion (with 95 % confidence limits) of noradrenaline (unbroken line) and adrenaline (broken line) in patients with toxemia of late pregnancy treated with reserpine during the first four days in hospital
Semi logarithmic scale

10.4±1.30 µg/24 hours. In the patients resting during the period of collecting the urine samples the noradrenaline excretion was 13.6±2.4 µg/24 hours.

In 11 patients the excretion of noradrenaline was less than 10 µg, the lowest value being 5.2 µg. Although six of these patients stayed at home, and one, No. 217, was occupied with physical work, no subjective

Table 22

Urinary excretion of noradrenaline and adrenaline in women given reserpine treatment in late toxemic pregnancy

Sample No.	Age (years)	Parity	Week of pregnancy	Body weight kg	Urine volume cc	NA µg/24 hours	A µg/24 hours	A %	NA µg/kg	A µg/kg	
105	25	II	37	65.0	1680	5.3	6.1	53	0.082	0.094	h
141	19	I	37	69.4	920	7.7	3.5	31	0.111	0.050	h
144	35	I	39	72.6	1210	19.4			0.267		r
152	23	II	38	64.0	1280	8.0	3.0	27	0.126	0.048	h
154	24	I	41	67.0	1320	6.5	2.8	30	0.097	0.042	h
157	34	I	37	75.9	1030	10.3	2.2	18	0.136	0.029	h
165	22	I	38	77.9	1500	14.0			0.180		r
184	21	I	36	69.5	1000	6.4	1.3	17	0.092	0.019	r
188	39	II	35	81.3	1100	7.5	3.5	32	0.092	0.043	r
187	31	II	40	69.9	1600	12.0	4.8	30	0.172	0.069	r
202	19	I	38	58.8	800	5.2	2.0	27	0.089	0.034	r
206	30	III	40	47.5	1550	13.1			0.275		r
213	30	II	40	68.0	1100	7.0	2.3	22	0.103	0.034	h
217	21	I	40	67.3	700	8.4	2.1	20	0.124	0.031	w
218	21	I	37	77.3	1240	9.0	3.3	27	0.116	0.043	h
228	32	III	39	73.5	1640	21.0	2.4	10	0.285	0.033	h
239	28	I	37	74.5	2100	13.4	4.7	26	0.180	0.063	h
271	21	I	30	48.5	1250	14.0	4.2	26	0.289	0.086	r
273	26	I	37	67.1	1020	13.0	2.6	18	0.193	0.039	h
278	26	IV	36	74.4	1300	10.5	6.8	40	0.141	0.092	r
284	37	VII	40	59.0	850	11.9	2.6	18	0.202	0.044	r
298	20	I	35	72.0	1000	20.8	4.4	24	0.290	0.062	w
312	21	I	37	63.0	1300	10.4	2.1	17	0.165	0.033	w
326	26	I	31	61.3	700	5.7	13.0	70	0.093	0.212	r
337	28	III	40	61.3	1400	13.0	12.0	48	0.211	0.197	r
345	40	II	36	97.5	2000	38.5	6.6	15	0.329	0.068	r
355	44	IV	35	102.5	[illegible]	19.0	3.4	15	0.152	0.027	r
Mean	27		37	69.5	1218	11.83	4.24	25.1	0.170	0.062	
SD					390	6.12	1.96	13.2	0.076	0.034	
SE					±80	±1.20	±0.44	±2.7	±0.015	±0.007	
n 27							27	24			

h = at home
r = resting
w = at work

symptoms were observed which might have been thought to be caused by the low noradrenaline excretion

The average adrenaline excretion in the patients with toxemic pregnancy given reserpine treatment was 4 2 ± 0 44 (1 3—13 0) µg/24 hours The amount of adrenaline excreted was 25 1 per cent

Calculated per kilogramme of body weight the 24 hour excretion of noradrenaline was 0 17 µg and that of adrenaline 0 06 µg

The average 24 hour output of urine by the patients in this group was rather small, 1218 ± 80 ml The average age was 27 (17—43) years, and the duration of pregnancy 37 (31—41) weeks

Effect of reserpine on the excretion of noradrenaline and adrenaline in the first four days of treatment in hospital

1 Material

As it was observed that the noradrenaline excretion in patients treated with reserpine in late toxemic pregnancy was less than in the patients with toxemia and not given reserpine, an attempt was made to

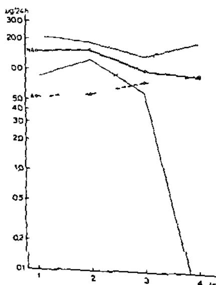


Figure 5

Average urinary excretion (with 95 % confidence limits) of noradrenaline (unbroken line) and adrenaline (broken line) in patients with toxemia of late pregnancy treated with reserpine during the first four days in hospital.
Semi logarithmic scale

find out how soon the decreasing effect of reserpine on the excretion appeared. The noradrenaline and adrenaline excretion was studied during the first four days of treatment in 12 patients admitted to hospital due to toxemia of pregnancy. Eleven of these patients were included in the group pre-eclampsia gravis and one in the group pre-eclampsia levis.

Reserpine treatment was started immediately on admission to hospital except in two instances when the patients had had reserpine for 10 days before admission. In general 0 9 mg was given daily *by mouth in the hospital*. Two patients with severe toxemic pregnancy were given two *intramuscular injections of 2 5 mg each immediately after admission*; the medication was continued as mentioned above.

2. *Results*

The average urinary excretion of noradrenaline decreased during reserpine medication (*Table 21, Fig. 5*), being 15 1 µg/24 hours before *starting the treatment, on the second day 15 4 µg/24 hours, on the third* 9 9 µg/24 hours and on the fourth day 8 7 µg/24 hours. At the same time a gradual increase occurred in the excretion of adrenaline: on the first day the content was 5 4 µg/24 hours, on the third 7 5 µg/24 hours and on the fourth day 9 0 µg/24 hours.

Before starting the treatment the average blood pressure of the patients with toxemia of pregnancy was 171/112 mmHg (Table 21, Fig. 3) but a small decrease gradually occurred: to 161/105 mmHg on the second day, to 155/102 mmHg on the third and to 150/101 mmHg on the fourth. Thus the blood pressure had not become normalized in four days. Likewise the proteinuria, which was 1 6 g/24 hours on the first day and 1 3 g/24 hours on the fourth (Fig. 4). In only one patient the blood pressure rose from 160/90 mmHg on the first day to 180/120 mmHg on the third in spite of the patient resting in hospital and being given daily doses of 0 9 mg of reserpine. The output of urine was 1260 ml on an average on the first day and 1330 ml/24 hours on the fourth. The average pulse rate remained unchanged, being 75 beats/minute on the first day and 72 beats/minute on the fourth.

Conclusions

The average noradrenaline excretion, 13 6 µg/24 hours, in the patients with toxemia of pregnancy resting in hospital and given reserpine did not differ significantly from that in the resting patients not given reserpine, 15 0 µg/24 hours (p > 0 05). But in the 13 patients with toxemic pregnancy occupied with housework or doing physical work during the

reserpine treatment, the excretion of noradrenaline, 10.4 µg/24 hours, was highly significantly less than in those working at home and not given reserpine, 24.0 µg/24 hours, and in those doing physical work, 26.3 µg/24 hours. On the basis of this comparatively small material it seems as if reserpine did not change the excretion at rest, but prevented the rise in noradrenaline excretion caused by activity.

Table 25

Diurnal rhythm of urinary excretion of noradrenaline and adrenaline in late normal pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Noradrenaline µg/12 hours		Adrenaline µg/12 hours		
					Day	Night	Day	Night	
64	24	III	28	65.0	27.3	7.7	3.3	0.6	h
66	24	II	32	65.0	16.6	8.3	2.0	0.8	h
68	20	I	31	61.0	12.6	8.4	1.7	1.2	h
70	20	I	38	73.5	11.1	5.7	1.9	0.5	w
72	23	I	32	64.0	12.8	3.5	3.2	2.1	h
74	20	I	36	94.0	9.2	3.1	3.7	1.2	h
87	26	II	41	67.0	7.2	4.0	1.3	1.1	h
93	30	I	33	74.6	19.0	7.4	1.0	0.5	w
96	32	II	32	66.0	11.2	6.0	2.0	1.2	w
99	33	III	36	78.0	29.7	2.9	4.1	2.4	h
108	26	V	34	76.0	13.1	5.4	5.4	3.2	h
111	24	II	36	72.0	26.0	7.8	14.2	3.7	h
114	24	III	36	67.0	13.8	9.6	3.2	1.0	h
117	21	I	37	66.5	25.0	6.5	9.7	2.9	h
120	24	I	38	64.0	19.5	5.9	2.8	1.5	h
123	20	II	36	80.5	13.4	5.1	1.2	0.9	h
129	26	I	33	61.8	14.0	3.2	2.7	1.5	h
179	26	II	35	59.0	9.1	6.0			h
233	26	II	38	76.0	5.5	5.5	1.6	3.3	h
236	21	I	36	75.5	10.1	4.8	3.7	2.0	h
294	19	I	34	66.0	6.7	6.7	2.1	1.7	h
329	23	I	39	66.0	10.2	3.5	2.2	1.6	h
331	18	I	38	74.2	11.2	6.4	5.0	7.6	h
339	23	II	35	59.5	5.7	7.1	5.1	5.0	h
341	20	II	38	76.5	26.4	9.7	4.1	2.2	h
381	32	V	39	89.9	4.7	5.2	1.6	0.8	r
Mean	24		35	70.6	13.98	5.87	3.50	2.02	
SD					7.44	1.94	3.00	1.69	
SF					±1.43	±0.38	±0.60	±0.32	
n 26					26	26	25	25	

w = at work
h = at home
r = resting in bed

On study of the decrease in *noradrenaline excretion during reserpine* treatment, it was observed that in the patients with normal pregnancy treated in hospital the excretion was 15.1 and 15.4 μg/24 hours in the first two days, likewise in the resting toxemic pregnant patients. On the third day there was a distinct decrease, however, to 9.9 μg/24 hours, which is less than in the patients with normal pregnancy resting in hospital, 13.7 μg/24 hours. Thus, the decreasing effect of reserpine on the urinary excretion of noradrenaline appeared on the third day of treatment.

The urinary excretion of adrenaline in patients with toxemia of pregnancy treated with reserpine, 4.2 μg/24 hours, was normal and only slightly less than in those with toxemia resting in hospital, 5.3 μg/24 hours; the *difference was not significant* ($p > 0.05$). *On administration* of reserpine to the toxemic women, the adrenaline excretion increased in the first three *days from 5.4 to 7.5 μg/24 hours, but the increase was not significant.*

Reserpine treatment alleviated the symptoms of toxemic pregnancy but complete disappearance of the symptoms was not achieved in four days. Since rest alone resulted in partial relief of the toxemic symptoms, and since the group of patients given reserpine and that of the patients resting and not given reserpine are not quite comparable as to the degree of toxemia of pregnancy, the role of reserpine in the alleviation of the symptoms of toxemia cannot be determined on the basis of this small material. The fact that the excretion of noradrenaline decreased on the third day of reserpine treatment seems, *however*, to point to *the action* of the drug, especially since the excretion of noradrenaline did not decrease *in the same degree in the patients with toxemic pregnancy resting* in hospital, but not given reserpine.

DIURNAL RHYTHM IN URINARY EXCRETION OF *NORADRENALINE AND ADRENALINE* IN LATE PREGNANCY

Normal pregnancy

1. Material

The urinary excretion of noradrenaline and adrenaline by day and night was studied separately in 26 women with normal pregnancy. Twenty-two of these were on maternity leave but occupied with housework, three were working as shop assistants, and one was resting in hospital due to premature rupture of the fetal membranes.

The day time excretion was determined in samples *collected* between 8 a.m. and 8 p.m., when the physical activity is greatest, and the excretion

by night in those taken between 8 p m and 8 a m The period for collecting the day time samples of the women doing physical work was calculated from the time of leaving home in the morning, and that for collecting the night samples was started 12 hours later

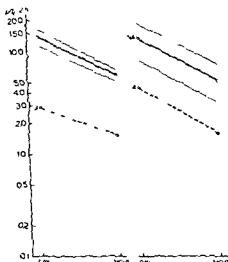


Figure 6
Diurnal variation (with 95% confidence limits) in urinary excretion of noradrenaline and adrenaline in late normal (left) and toxemic (right) pregnancy
Semi logarithmic scale

2 *Results*

The average day time excretion of noradrenaline was 14 0 ± 1 43 (4 7—29 7) µg/12 hours (Table 23 Fig 6) The corresponding *excretion* by night was 5 9 ± 0 38 (2 9—9 7) µg/12 hours The day time excretion of adrenaline was 3 5 ± 0 60 (1 0—14 2) µg/12 hours The average *excretion of* adrenaline by night decreased to 2 0 ± 0 32 (0 5—7 6) *µg/12 hours*

The day time output of urine was 570 (350—1200) ml, and that by night 655 (300—1360) ml

Toxemic pregnancy

1 *Material*

The excretion of noradrenaline and *adrenaline* was determined in 13 women with toxemic pregnancy Three of these belonged to the group pre eclampsia gravis and the remainder *to that* of pre-eclampsia levis

Five of the women studied were occupied with housework, five were treated in hospital owing to toxemia of pregnancy and three had employment requiring considerable physical activity. One of the patients, No 105, who was resting in hospital, received daily doses of 0.3 mg of reserpine for 80 days before collection of the samples was started.

2 *Results*

The average day time excretion of noradrenaline in the urine in late toxemic pregnancy was 13.6 ± 3.10 (2.5—42.0) μg/12 hours and the excretion by night 5.1 ± 1.13 (1.6—18.0) μg/12 hours (Table 24, Fig 6). In the eight women working at home or doing physical work, the average day time excretion was 18.7 μg/12 hours and the excretion by night 6.3 μg/12 hours. In the women resting in hospital, the average day time excretion was 5.6 μg/12 hours and the average excretion by night 3.0 μg/12 hours. Thus the rhythm of noradrenaline excretion was distinctly retained in

Table 24

Diurnal rhythm of urinary excretion of noradrenaline and adrenaline in late toxemic pregnancy

Sample No	Age (years)	Parity	Week of pregnancy	Body weight kg	Noradrenaline μg/12 hours		Adrenaline μg/12 hours		
					Day	Night	Day	Night	
62	22	I	28	86.0	42.0	18.0	7.3	0.3	w
76	28	I	31	77.5	10.5	4.7	3.0	2.8	w
78	21	I	28	67.5	18.0	5.0	2.6	1.4	w
90	34	III	28	64.2	29.6	4.4	3.0	2.3	h
102	22	I	37	62.0	12.0	6.7	5.5	1.6	h
105	25	II	37	65.0	3.7	1.6	5.6	0.2	rx
126	24	I	39	69.5	13.2	3.0	2.5	1.2	h
335	17	I	38	67.0	9.7	4.5	7.0	2.7	h
337	22	I	38	73.5	14.2	4.0	3.3	3.0	h
375	18	I	40	59.0	6.0	3.0	1.1	1.5	r
377	26	I	39	67.0	2.5	2.5			r
379	26	I	39	67.0	5.2	[illegible]			r
383	30	V	39	75.0	10.6	4.4	3.9	0.7	r
Mean	24		35	69.2	13.60	5.06	4.10	1.60	
SD				10.1	11.40	4.06	1.96	1.00	
SE					±3.10	±1.13	±0.59	±0.30	
n 13					13	13	11	11	

w = at work
h = at home
r = resting in bed
x = reserpine treatment

the patients with toxemia of pregnancy, whether they were doing housework or physical work. This was also the case, to some extent, in those resting in hospital, even if the difference between their day and night excretion decreased markedly.

The average day time excretion of adrenaline in the patients with toxemia of pregnancy was 4.1 ± 0.59 (1.4—7.3) μg/12 hours. The corresponding excretion by night was 1.6 ± 0.30 (0.2—3.0) μg/12 hours.

The average day time output of urine was 740 (300—1180) ml/12 hours and the output by night 600 (200—1050) ml/12 hours.

Conclusions

The day time excretion of noradrenaline in late normal and toxemic pregnancy was about 2.5 times greater than the excretion by night. The day time excretion of adrenaline was likewise greater than that by night, being 1.7 times greater than the excretion by night in late normal pregnancy and 2.6 times greater than in late toxemic pregnancy.

Five of the women studied were occupied with housework, five were treated in hospital owing to toxemia of pregnancy and three had employment requiring considerable physical activity. One of the patients, No. 105, who was resting in hospital, received daily doses of 0.3 mg of reserpine for 80 days before collection of the samples was started.

2. *Results*

The average day time excretion of noradrenaline in the urine in late toxemic pregnancy was 13.6 ± 3.10 (2.5—42.0) μg/12 hours and the excretion by night 5.1 ± 1.13 (1.6—18.0) μg/12 hours (Table 24, Fig. 6). In the eight women working at home or doing physical work, the average day time excretion was 18.7 μg/12 hours and the excretion by night 6.3 μg/12 hours. In the women resting in hospital, the average day time excretion was 5.6 μg/12 hours and the average excretion by night 3.0 μg/12 hours. Thus the rhythm of noradrenaline excretion was distinctly retained in

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Diurnal rhythm of urinary excretion of noradrenaline and adrenaline in late toxemic pregnancy

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62	22	I	28	86.0	42.0	18.0	7.3	0.3	w
76	28	I	31	77.5	10.5	4.7	3.0	2.8	w
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90	34	III	28	64.2	29.6	4.4	3.0	[illegible].3	h
102	22	I	37	62.0	12.0	6.7	5.5	1.6	h
105	25	II	37	65.0	3.7	1.6	5.6	0.2	rx
126	24	I	39	69.5	13.2	3.0	2.5	1.[illegible]	h
335	17	I	38	67.0	9.7	4.5	7.0	[illegible].7	h
337	22	I	38	73.5	14.2	4.0	3.3	3.0	h
375	18	I	40	59.0	6.0	3.0	1.4	1.5	r
377	26	I	39	67.0	2.5	2.5			r
379	26	I	39	67.0	5.2	3.[illegible]			r
383	30	V	39	75.0	10.6	4.4	3.9	0.7	r
Mean	24		35	69.2	13.60	5.06	4.10	1.10	
SD				10.1	11.40	4.06	1.96	1.00	
SE					±3.10	±1.13	±0.59	±0.30	
n 13					13	13	11	11	

w = at work
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the patients with toxemia of pregnancy, whether they were doing house work or physical work. This was also the case to some extent, in those resting in hospital even if the difference between their day and night excretion decreased markedly.

The average day time excretion of adrenaline in the patients with toxemia of pregnancy was 4.1 ± 0.59 (1.4—7.3) μg/12 hours. The corresponding excretion by night was 1.6 ± 0.30 (0.2—3.0) μg/12 hours.

The average day time output of urine was 740 (300—1180) ml/12 hours and the output by night 600 (200—1050) ml/12 hours.

Conclusions

The day time excretion of noradrenaline in late normal and toxemic pregnancy was about 2.5 times greater than the excretion by night. The day time excretion of adrenaline was likewise greater than that by night, being 1.7 times greater than the excretion by night in late normal pregnancy and 2.6 times greater than in late toxemic pregnancy.

DISCUSSION

In the present investigation, the excretion of free noradrenaline and adrenaline was determined in the urine of women in late normal or toxemic pregnancy. The study was performed under different environmental conditions, i.e., the pregnant women were grouped according to their occupation: housework, physical work away from home, resting in hospital, or undergoing treatment with reserpine. Division of the material was considered necessary in order to evaluate the strain of pregnancy as such on the organism, and its reaction to bodily strain and rest.

Urinary excretion of noradrenaline and adrenaline in women occupied with housework.

a) *Late normal pregnancy.* In this group, 105 women at the thirty-fifth week of pregnancy, on an average, were studied. Their 24 hour excretion of noradrenaline was 19.7 µg/24 hours and that of adrenaline 4.4 µg. In the controls the noradrenaline excretion was 23.8 µg/24 hours and that of adrenaline 5.2 µg/24 hours. On comparison of these results with those obtained in other investigations (KARKI 1956) of the noradrenaline excretion in women subjected to the corresponding strain, it was established that, using biological methods, the average noradrenaline excretion recorded was 22.9 µg/24 hours and that of adrenaline 4.2 µg. On the control days before a major surgical operation, the noradrenaline excretion was 21.7 µg/24 hours and that of adrenaline 3.7 µg/24 hours (HAMER *et al.* 1957), and in a group of 26 healthy women students prior to matriculation examination the excretion of noradrenaline was 15.3 µg/24 hours and that of adrenaline 3.5 µg/24 hours (PEKKARINEN *et al.* 1961). It is thus evident that pregnancy does not strain the organism in a manner that might increase the urinary excretion of noradrenaline or adrenaline. My observation corroborates the view (BURN 1953, RAAB & GIGEE 1954, SUBRAHMANYAM 1959) that the noradrenaline and adrenaline content in urine does not rise in connection with normal pregnancy. The catechol content increases distinctly, however, in the last trimester as verified by tests performed according to the spectrophotometric method (OESTERLING *et al.* 1962).

In exceptional cases, the noradrenaline excretion increases to 570 µg/24 hours and the adrenaline excretion to 260 µg/24 hours due to such exceptional stress as thermal burn (GOODALL *et al.* 1957). Cardiac infarction increases the excretion of noradrenaline to about 100 µg/24 hours

partly because of the shock sustained (FORSSMAN *et al* 1952) Owing to major surgical interference such as thoracal or abdominal operations, the noradrenaline excretion rises in women to twice the pre operative amount, being on the second post-operative day on an average 38 8 μg/24 hours in women and 71 2 μg/24 hours in men The adrenaline excretion rises to about three times that amount, in women to 9 9 μg/24 hours and in men to 14 4 μg/24 hours on an average, in men the increase is thus considerably greater (HALME *et al* 1957) In a group of students about to pass the matriculation examination the adrenaline excretion rose about six times in women, or, on an average to 19 6 μg/24 hours In one fourth of the students the excretion exceeded 30 μg/24 hours The highest adrenaline level was however, recorded in a male student of this group, 100 8 μg/24 hours In all the excretion of noradrenaline remained unchanged (PEKKARINEN *et al* 1961) Owing to the effect of medium cardiac insufficiency, the excretion of adrenaline was 11 7 μg/24 hours on the first day of treatment in hospital (PEKKARINEN *et al* 1960 a) No increased excretion of noradrenaline and adrenaline during normal pregnancy comparable with the above mentioned values was observed in the present investigation On the other hand, it should be noted that the excretion of adrenaline increased only slightly during labour, and at the same time the noradrenaline excretion decreased slightly, according to GEMZELL *et al* (1956)

In general under strenuous conditions considerable individual variations occurred in the excretion of noradrenaline and adrenaline For instance after major surgical operations the noradrenaline excretion in 8 of 36 women increased their average excretion being over 50 μg/24 hours on the first three post operative days and on some single day almost 150 μg At the same time the average adrenaline content in 9 of 30 women was over 10 μg/24 hours on the first three post operative days and on some days over 40 μg maximum 61 0 μg On the other hand, in numerous urine samples of these women, the adrenaline content was almost nil (HALME *et al* 1957) In general, the adrenaline excreted in the women of the group of students studied during the period of matriculation was increased, in exceptional cases to 56 8 μg/24 hours, and in numerous instances to 30 μg or over (PEKKARINEN *et al* 1961)

Attention should be paid to the fact that in the present investigation, the individual noradrenaline and adrenaline content varied slightly, and no clearly increased excretion was observed In general, the 24 hour excretion of noradrenaline is rarely over 35 μg/24 hours and less than 12 μg 24 hours and the adrenaline excretion does not as a rule exceed 8 μg/24 hours In the present material, there were only four women with a noradrenaline excretion exceeding 35 μg/24 hours the greatest amount being only slightly increased 60 0 μg/24 hours, and in nine women the nor

DISCUSSION

In the present investigation, the excretion of free noradrenaline and adrenaline was determined in the urine of women in late normal or toxemic pregnancy The study was performed under different environmental conditions, i e, the pregnant women were grouped according to their occupation housework, physical work away from home, resting in hospital or undergoing treatment with reserpine Division of the material was considered necessary in order to evaluate the strain of pregnancy as such on the organism, and its reaction to bodily strain and rest

Urinary excretion of noradrenaline and adrenaline in women occupied with housework

a) *Late normal pregnancy* In this group, 105 women at the thirty fifth week of pregnancy, on an average, were studied Their 24 hour excretion of noradrenaline was 19 7 µg/24 hours and that of adrenaline 4 4 µg In the controls the noradrenaline excretion was 23 8 µg/24 hours and that of adrenaline 5 2 µg/24 hours On comparison of these results with those obtained in other investigations (KARKI 1956) of the noradrenaline excretion in women subjected to the corresponding strain, it was established that, using biological methods, the average noradrenaline excretion recorded was 22 9 µg/24 hours and that of adrenaline 4 2 µg On the control days, before a major surgical operation, the noradrenaline excretion was 21 7 µg/24 hours and that of adrenaline 3 7 µg/24 hours (HAMMI *et al* 1957), and in a group of 26 healthy women students prior to matriculation examination, the excretion of noradrenaline was 15 3 µg/24 hours and that of adrenaline 3 5 µg/24 hours (PEKKARINEN *et al* 1961) It is thus evident that pregnancy does not strain the organism in a manner that might increase the urinary excretion of noradrenaline or adrenaline My observation corroborates the view (BURN 1953, RAAB & GIGEE 1954, SUBRAHMANYAM 1959) that the noradrenaline and adrenaline content in urine does not rise in connection with normal pregnancy The catechol content increases distinctly however, in the last trimester as verified by tests performed according to the spectrophotometric method (OESTERLING *et al* 1962)

In exceptional cases, the noradrenaline excretion increases to 570 µg 24 hours and the adrenaline excretion to 260 µg 24 hours due to such exceptional stress as thermal burn (GOODALL *et al* 1957) Cardiac infarction increases the excretion of noradrenaline to about 100 µg/24 hours

in order to clarify the effect of physical exertion on the excretion of noradrenaline and adrenaline in late normal pregnancy, the average content of the former substance was 24 0 µg/24 hours and that of the latter 4 9 µg These average values must be considered normal as they agree with those published in numerous other reports on the same subject In the present material the excretion of noradrenaline in women doing physical work was significantly higher than in those occupied with housework during late normal pregnancy, but the difference in the adrenaline excretion between these two groups of women was not significant The individual variations in the excretion of noradrenaline and adrenaline were exceedingly small also in this group No average values over twice the normal for noradrenaline excretion were recorded The largest amount of noradrenaline excreted 42.5 µg/24 hours, should be considered normal, likewise the largest amount of adrenaline excreted, 8 0 µg/24 hours

In the women doing light physical work in late toxemic pregnancy, the excretion of noradrenaline, 26 3 µg/24 hours, as well as that of adrenaline 5 9 µg/24 hours was slightly higher than in any of the other groups in the present study, all the values being within normal ranges however These values for urinary excretion of the two substances were only slightly and not significantly higher than the corresponding values for the women doing housework in late normal pregnancy It should be pointed out that, due to the effect of about similar exertion, the increase in the urinary excretion of noradrenaline was greater in late normal pregnancy than in late toxemic pregnancy Only small variations were noted in the individual excretion of noradrenaline and adrenaline in women with toxemia of pregnancy doing light physical work in one only was the noradrenaline excretion slightly raised, 55 0 µg/24 hours and in two the adrenaline content was slightly increased, 9 5 µg/24 hours and 12 8 µg/24 hours

In the present investigation there was no group of women doing heavy physical work which might endanger the course of pregnancy It may be assumed however that the strain of an eight hour working day of a shop-assistant of a factory hand or of others with occupations mentioned previously in connection with increased weight, about 10 kg toward the end of pregnancy and the enlarged uterus encumbering movements must, in all cases be considered moderate bodily strain

Owing to the effect of physical work the urinary excretion of noradrenaline and adrenaline increased only 1 1 to 1 3 times in late normal and toxemic pregnancy compared with the amounts of these substances excreted in women doing housework In healthy people, the noradrenaline excretion rises four to six times on physical activity, for instance, in athletes in connection with training for sports (KÄRKI 1956), three times

adrenaline excretion was less than the above mentioned 12 μg/24 hours. *In only eight women of this rather large series the excretion of adrenaline was over 8 μg/24 hours.*

The duration of normal pregnancy did not seem to affect the urinary excretion of noradrenaline and adrenaline unfavourably. Before the 32nd week of pregnancy the noradrenaline excretion 21.9 μg/24 hours was *somewhat greater than between the 37th and the 40th week 17.9 μg/24 hours.* The reverse observation was made with regard to adrenaline excretion before the 28th week 3.6 μg/24 hours which is slightly less than during the last month of pregnancy 4.6 μg/24 hours.

In late toxemic pregnancy the 24 hour excretion of noradrenaline was *24.1 μg and that of adrenaline 4.5 μg* in women with comparatively mild toxemia of pregnancy occupied with housework. The noradrenaline level was thus significantly increased in toxemic pregnancy, compared with that in normal pregnancy. In spite of this neither the average 24 hour excretion of noradrenaline nor the majority of the individual amounts *of noradrenaline excreted are according to general conception greater* than those excreted in non pregnant women. In the present material only 5 of the 36 determinations of the 24 hour excretion of noradrenaline exceeded 40 μg/24 hours, the largest amount being 80 μg/24 hours. The excretion of adrenaline was over 8 μg/24 hours, maximum 16.0 μg, in only 4 of the 37 *patients* studied.

In a series including seven patients with eclampsia and numerous with severe toxemia of pregnancy SUBRAHMANYAM (1959) noted an average of 68.3 μg/24 hours in the urinary excretion of noradrenaline whereas in *late* normal pregnancy the excretion was 36.2 μg/24 hours. The adrenaline excretion in late toxemic pregnancy, 10.4 μg/hours was about twice that recorded toward the end of normal pregnancy 6.5 μg/24 hours. BURN (1953) on the other hand observed no increase in the total excretion of noradrenaline in six women in *late toxemic pregnancy compared with* that in seven in late normal pregnancy. Nor was the noradrenaline content in the plasma higher in five women suffering from toxemia of pregnancy than that in 30 women with normal pregnancy (HOCHULI 1960).

The results obtained in the present investigation showed that during the last three months *of toxemic pregnancy the excretion* of noradrenaline decreased slightly. In the eighth month the excretion was 33.5 μg/24 hours and in the tenth 21.6 μg/24 hours but as only few determinations were made in the eighth month the result were regarded *as doubtful. The* cause of reduced excretion of noradrenaline in such a short period must *be considered* due *to* women approaching term resting more than usually.

Effect of physical exertion on the excretion of noradrenaline and adrenaline in late normal and toxemic pregnancy. In 14 women studied

with toxemic pregnancy than in those with normal pregnancy and no raised noradrenaline levels were observed the excretion ranged between 7 0 and 30 5 µg/24 hours

The urinary excretion of adrenaline in the women resting during late normal pregnancy, 5 1 µg/24 hours or toxemic pregnancy, 5 3 µg/24 hours remained on the same level as that in the women doing housework and physical work or in non pregnant women The amount of adrenaline excreted in three women resting in late normal pregnancy and that in one woman resting in late toxemic pregnancy exceeded 10 µg/24 hours

While the average adrenaline percentage in women toward the end of normal and toxemic pregnancy was less than 20 per cent in those doing housework or physical work that in women resting toward the end of normal pregnancy was 25 6 per cent and in women resting toward the end of toxemic pregnancy 26 5 per cent This was due to the noradrenaline excretion decreasing distinctly during rest whereas the adrenaline excretion remained unchanged In four cases almost half of the amount of all the biologically active noradrenaline and adrenaline excreted in the urine consisted of adrenaline

In recumbent position when the regulation of the blood pressure does not require as high a sympathetic tone as in erect position the urinary excretion of noradrenaline and adrenaline was low, the average noradrenaline excretion being 0 59—0 99 µg/hour in recumbent position and 1 85—3 35 µg/hour on tilting to an angle of 75° head up (v EULER *et al* 1955 b SUNDIN 1956 1958) this corresponds completely with the amount excreted by women resting as observed in the present study

The adrenaline excretion in resting healthy people varies between 0 11 and 0 27 µg/hour (v EULER *et al* 1955 b SUNDIN 1956 1958) In the present study the average excretion of adrenaline in late normal or toxemic pregnancy was 0 22 µg/hour and no difference was observed between the pregnant and the non pregnant women in this respect

In a series of patients with hypertension (SUNDIN 1958) the noradrenaline excretion recorded was 0 56 µg/hour in connection with rest, or the same as that in healthy persons and in pregnant women and that of adrenaline 0 47 µg/hour or twice as much as in women with normal or toxemic pregnancy

Effect of reserpine treatment on the urinary excretion of noradrenaline and adrenaline The average urinary excretion of noradrenaline in 27 patients with toxemia of pregnancy treated with reserpine was 11 8 µg/24 hours, which is significantly less than that in the toxemic patients doing housework or physical work but did not differ from the noradrenaline excretion in toxemic patients resting in hospital An observation of interest was that the noradrenaline excreted in 13 patients with toxemic preg

due to the effect of muscular exercise on the ergometer (TAKAHASHI 1961) and almost ten times owing to very considerable physical effort and strain such as the Marathon race (KARKI 1956). The *excretion of adrenaline increases to the double in association with physical activity* (TAKAHASHI 1961) and in a short while four to five times in connection with examinations (PEKKARINEN *et al.* 1961). It thus seems permissible *to state that in pregnant women the organism does not react to physical activity by an equal increase in noradrenaline excretion as in non-pregnant women and that pregnancy is not*, at least not constantly, such a period of tension that would cause the excretion of adrenaline to increase. It is well known that, *owing to the effect of an extensive surgical operation, the increase in noradrenaline and adrenaline excretion is less in women* compared with men (HALMI *et al.* 1957).

Since physical work did not increase the amount of *noradrenaline and adrenaline excreted in women in late pregnancy to the same extent as that excreted in non-pregnant women*, it may be assumed that in the former the high corticosteroid content in the plasma and the abundance of extracellular fluid decreased the action of the neurogenic *mechanism on the regulation of the blood pressure* (PEKKARINEN 1962). Another possibility is that the organism is protected by suppression of the liberation of noradrenaline and adrenaline from the sympathetic nerve endings by the high content of free corticosteroids in the plasma and by retention of sodium chloride and fluid.

It is generally known that physical activity aggravates the symptoms of toxemic pregnancy. But, as it was observed in the present study that neither the excretion of noradrenaline nor that of adrenaline increased considerably in late normal and toxemic pregnancy in women doing physical work, it may be concluded that the increase in these substances are not directly responsible for the impairment.

Effect of rest on the noradrenaline and adrenaline excretion during normal and toxemic pregnancy. Owing to rest the excretion of noradrenaline decreased significantly in late normal and toxemic pregnancy compared with that observed in the corresponding groups of women doing housework. The average amount of noradrenaline excreted in patients toward the end of normal pregnancy was 13.7 μg/24 hours or 0.57 μg/hour, whereas that in the women occupied with housework was 19.7 μg/24 hours. In not a single instance was an increased excretion noted, the greatest 24 hour excretion was only 24.8 μg/24 hours.

The average noradrenaline excretion in the women resting toward the end of toxemic pregnancy was 15.5 μg/24 hours or 0.64 μg/hour, while that in the women occupied with housework was 24.0 μg/24 hours. Thus the noradrenaline excretion decreased due to rest somewhat more in the women

investigation, the increased adrenaline excretion in patients given reserpine was not due to the drug. On the basis of this observation, it may sooner be said that reserpine at least not with the dose given affected the adrenaline secretion of the adrenal glands as the reaction of the glands to the stress of admittance to hospital was not inhibited.

The noradrenaline excretion in patients with toxemia of pregnancy resting in hospital towards the end of pregnancy decreased due to administration of reserpine whereas in those not given the drug there seemed sooner to be a slight increase.

On intramuscular injection of 15 mg of reserpine, the urinary excretion of noradrenaline and adrenaline has been observed to decrease in man (GADDUM *et al.* 1958). In test animals 1 mg/kg of reserpine has been noted to increase the urinary excretion of adrenaline about six times at the beginning of the treatment (DE JONGH 1958) but it rapidly returns to the initial level (CARLSSON *et al.* 1957). During long term administration of reserpine to rabbits the adrenaline content in the tissues decreased to 10 per cent in ten days, but later there was a tendency to increased values (HAGGENDAL & LINDQVIST 1963). On the other hand, reserpine treatment for 6 to 45 months with doses of 1—9 mg did not change the urinary excretion of adrenaline in man (CARLSSON *et al.* 1959 a). This observation is confirmed by the results obtained in the present study. When daily doses of 0.2—0.9 mg of reserpine were given to mothers for two to 60 days prior to parturition the average urinary noradrenaline and adrenaline excretion in the newborn did not differ from that in the newborn of mothers with normal pregnancy or untreated toxemic pregnancy (CASTRÉN *et al.* 1963).

The dose of reserpine 0.3—0.9 mg/day given to the patients studied in the present investigation is rather small compared with that given to test animals 1—5 mg/kg (BERTLER *et al.* 1956, CARLSSON *et al.* 1956, PAASONEN & KRAYER 1957, KRONEBERG & SCHUMANN 1958). However in the tissue of test animals the catecholamines have been observed to decrease in six to 16 hours after doses of 0.005—0.1 mg/kg of reserpine (CARLSSON *et al.* 1957, PEKKARINEN *et al.* 1958). Not until 0.5 mg/kg of reserpine had been given was it possible to bring down the noradrenaline and adrenaline content in the adrenals to half of the previous amount (CARLSSON *et al.* 1957). Since the daily dose of reserpine used in the present study was 0.004—0.013 mg/kg calculated on the patients' average weight it is possible that this dose given over a short period would not affect the adrenal glands. This would explain the fact that the urinary excretion of adrenaline in the toxemic patients was not reduced due to reserpine treatment. Among the patients treated with reserpine there were some with toxemia of pregnancy who had received the drug without interruption for almost three

nancy belonging to this group, who were treated with reserpine and doing housework or physical work, was highly significantly less than that of the women not given reserpine and doing equally strenuous work. But reserpine did not reduce the noradrenaline excretion in the patients with toxemia of pregnancy resting in hospital. *Augmentation of the noradrenaline excretion caused by increased physical activity was thus prevented by reserpine treatment.* This blocking effect on the excretion of noradrenaline explains the favourable clinical action of reserpine also on the physically active patients with toxemia of pregnancy.

In some patients, reserpine decreased the excretion of noradrenaline very considerably, as much as to 5.2 μg/24 hours. On study of the whole *group of patients treated with reserpine, the 24-hour excretion of noradrenaline* was observed to vary slightly from one subject to another, and no raised levels were noted.

The average 24-hour excretion of adrenaline in the toxemic patients treated with reserpine was 4.2 μg, whereas those not thus treated excreted 5.3 μg/24 hours. The difference between these two values was not significant. In the toxemic patients belonging to this group, doing housework or physical work, *and undergoing reserpine treatment, the adrenaline* excretion was 3.5 μg/24 hours and that in the patients resting 5.6 μg. On comparison of these values for excretion of adrenaline with those for the patients not given reserpine and exposed to an equal degree of exertion, it was observed that the values for the patients resting, but not given reserpine, 5.3 μg/24 hours, were the same as for those treated *with reserpine*. But the amount of adrenaline excreted by women with toxemic pregnancy doing housework and not given reserpine, 5.1 μg/24 hours, was slightly greater than that excreted in women given reserpine and working under similar conditions. The present material is too small, however, to establish definitely that the decreased adrenaline excretion was due to reserpine treatment.

In patients with toxemia of pregnancy, treated in hospital, and generally given doses of 0.9 mg of reserpine, the urinary adrenaline excretion increased during the first four days being 5.4 μg/24 hours on the first day and 9.0 μg on the fourth. The excretion increased on the first days also in the women with normal pregnancy admitted to hospital owing to premature rupture of the fetal membranes or to *prolonged pregnancy*. Even in the cases of healthy persons admittance to hospital is known *to cause a certain stress resulting in augmented excretion of adrenaline to* about double the initial amount. Under the same conditions only a slight increase in the excretion of noradrenaline was observed which like the adrenaline excretion returned to normal *on the seventh day in hospital* (TOLSON *et al.* 1962). It is thus evident that, as observed in the present

investigation the increased adrenaline excretion in patients given reserpine was not due to the drug. On the basis of this observation it may sooner be said that reserpine at least not with the dose given affected the adrenaline secretion of the adrenal glands, as the reaction of the glands to the stress of admittance to hospital was not inhibited.

The noradrenaline excretion in patients with toxemia of pregnancy resting in hospital towards the end of pregnancy decreased due to administration of reserpine, whereas in those not given the drug there seemed sooner to be a slight increase.

On intramuscular injection of 15 mg of reserpine the urinary excretion of noradrenaline and adrenaline has been observed to decrease in man (GADDUM *et al.* 1958). In test animals, 1 mg/kg of reserpine has been noted to increase the urinary excretion of adrenaline about six times at the beginning of the treatment (DE JONGH 1958) but it rapidly returns to the initial level (CARLSSON *et al.* 1957). During long term administration of reserpine to rabbits the adrenaline content in the tissues decreased to 10 per cent in ten days but later there was a tendency to increased values (HAGGENDAL & LINDQVIST 1963). On the other hand reserpine treatment for 6 to 45 months with doses of 1—3 mg did not change the urinary excretion of adrenaline in man (CARLSSON *et al.* 1959 a). This observation is confirmed by the results obtained in the present study. When daily doses of 0.3—0.9 mg of reserpine were given to mothers for two to 60 days prior to parturition the average urinary noradrenaline and adrenaline excretion in the newborn did not differ from that in the newborn of mothers with normal pregnancy or untreated toxemic pregnancy (CASTRÉN *et al.* 1963).

The *dose* of reserpine 0.3—0.9 mg/day given to the patients studied in the present investigation is rather small compared with that given to test animals 1—5 mg/kg (BERTLER *et al.* 1956, CARLSSON *et al.* 1956, PAASONEN & KRAYER 1957, KRONEBERG & SCHUMANN 1958). However in the tissue of test animals the catecholamines have been observed to decrease in six to 16 hours after doses of 0.005—0.1 mg/kg of reserpine (CARLSSON *et al.* 1957, PEKKARINEN *et al.* 1958). Not until 0.5 mg/kg of reserpine had been given was it possible to bring down the noradrenaline and adrenaline content in the adrenals to half of the previous amount (CARLSSON *et al.* 1957). Since the daily dose of reserpine used in the present study was 0.004—0.013 mg/kg calculated on the patients average weight it is possible that this dose given over a short period would not affect the adrenal glands. This would explain the fact that the urinary excretion of adrenaline in the toxemic patients was not reduced due to reserpine treatment. Among the patients treated with reserpine there were some with toxemia of pregnancy who had received the drug without interruption for almost three

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months. No difference was observed in the amount of adrenaline excreted, compared with that of the patients in other groups of the present investigation. It seems evident that not even an increased dose of reserpine would have brought about any changes in the excretion of adrenaline. With regard to the excretion of noradrenaline, the doses of reserpine used must likewise be considered adequate as no significant decrease was observed in spite of the doses being 1—9 mg/day (CARLSSON *et al.* 1959 a).

The period of reserpine treatment in the present investigation varied between three and 80 days. In test animals, the noradrenaline and adrenaline content in the tissue decreased distinctly as soon as half an hour after an intravenous injection of reserpine. The almost complete disappearance of noradrenaline and adrenaline from the tissue in one to 16 hours was considered to be due to the type of tissue (CARLSSON *et al.* 1957, PEKKARINEN *et al.* 1958). In rabbits, the adrenaline content in the plasma increased to twice or three times the normal in one half to four hours after intramuscular injection of 4 mg/kg of reserpine (KRONEBERG & SHUMANN 1958).

Oral administration of reserpine caused a distinct decrease in the noradrenaline content in the plasma in man on the third day after starting treatment (BURGER 1957). In the present study it was also observed that the noradrenaline excreted in the patients with toxemia of pregnancy, given reserpine immediately on admission to hospital, remained unchanged on the first two days, but on the third day, the level decreased, and later there was only a slight further decrease. It is thus evident that reserpine treatment for three days — the shortest period of reserpine therapy in the present investigation — must be considered sufficient before taking urine samples for determination of the noradrenaline and adrenaline levels.

It has been previously observed that large doses 15 mg of reserpine i.m. has a decreasing action on diuresis (GADDUM *et al.* 1958). Tests with 1—9 mg administered daily by mouth to schizophrenics did not inhibit diuresis, however (CARLSSON *et al.* 1959 a). After small therapeutic doses of reserpine as used in the present investigation, the average output of urine of the patients with toxemia of pregnancy, resting in hospital and not given reserpine, was 943 ml/24 hours and that of the patients with mild toxemia doing physical work 1630 ml/24 hours. The average urinary output of the patients with toxemia of pregnancy treated with reserpine was 1218 ml/24 hours which does not differ significantly from that of the patients in the groups mentioned. The urinary output of the toxemic pregnant patients resting in hospital but not given reserpine increased in four days from 955 ml/24 hours to 1180 ml, and the output of those given reserpine from 1260 ml/24 hours to 1330 ml. Although slightly increased diuresis may be merely a normal variation, it may be said that in the present material reserpine did not seem to have an inhibiting effect on diuresis.

If an excessive increase in body weight is the only symptom of toxemia of pregnancy or if in addition the blood pressure is only slightly raised treatment is started with drugs which increase diuresis. Hypotensive drugs usually reserpine are combined with diuretics if the blood pressure continues to rise. In the present investigation only three toxemic patients Nos 188, 273 and 278 (Table 22), received reserpine as well as diuretics chlorothiazide or hydrochlorthiazide. The average excretion of noradrenaline in these patients 10.3 µg/24 hours and of adrenaline 4.4 µg/24 hours does not differ from that in the patients treated with reserpine alone. In test animals it has been observed that if 2 mg/kg of hydrochlorthiazide is given two hours before an injection of reserpine there is no decrease in the adrenaline content in the adrenals which generally occurs if only reserpine is administered (COREP *et al* 1960). In patients with toxemia of pregnancy the action of reserpine and hydrochlorthiazide was converse in this respect. Clinically, the combination of these two substances yield good results (HOCHULI 1961).

Diurnal rhythm of noradrenaline and adrenaline excretion. The present material was divided into two 12 hour parts for study of the 24 hour rhythm of noradrenaline and adrenaline excretion: the day time rhythm was generally determined between 8 a.m. and 8 p.m., the period of physical activity, and the night rhythm between 8 p.m. and 8 a.m., which is the period of rest and sleep. Since the period of sleep covers 8 to 9 hours there remain 3 to 4 hours when the women studied were probably doing their own housework. The period for study of the 24 hour variation was divided into two parts instead of the usual three 8 hour periods as collection of the urine samples during several periods would afford certain technical difficulties.

The average urinary excretion of noradrenaline during the period of *rest and sleep* was 0.49 µg/hour in late normal pregnancy and 0.43 µg/hour in late toxemic pregnancy. The difference between these figures was not significant. In healthy people the noradrenaline excretion during the period of rest is 0.28—0.71 µg/hour (v. EULER *et al* 1955a, v. EULER *et al* 1955b, KARKI 1956, JANUSZEWICZ & WOCIAL 1960). Thus the excretion of noradrenaline in late normal and toxemic pregnancy varies between normal limits during the period of sleep and rest.

The average urinary excretion of adrenaline in late normal pregnancy was 0.17 µg/hour and in late toxemic pregnancy 0.13 µg/hour. In healthy people the excretion of adrenaline by night ranged between 0.07 and 0.14 µg/hour (v. EULER 1955a, v. EULER 1955b, KARKI 1956, JANUSZEWICZ & WOCIAL 1960). The adrenaline excretion during the period of rest and sleep must likewise be considered to vary between normal limits.

The *day time* amount of noradrenaline and adrenaline excreted in the urine depends on the strain of the work performed. The average excretion

of noradrenaline in healthy persons occupied with ordinary light routine work varies between 0.74 and 1.53 μg/hour, and that of adrenaline between 0.19 and 0.39 μg/hour (v. EULER *et al.* 1955 a, KÄRKI 1956, JANUSZEWICZ & WOCIAL 1960). These values correspond with those obtained for noradrenaline excretion, 1.16 μg/hour, and for adrenaline excretion, 0.29 μg/hour, in late normal pregnancy.

Thus the day time excretion in patients with normal pregnancy did not differ from that in healthy people subjected to the same degree of of excretion. But, in late toxemic pregnancy the noradrenaline excreted by eight women doing light housework was 1.50 μg/hour and that of adrenaline 0.37, or slightly more than in late normal pregnancy. The excretion of noradrenaline by night in late toxemic pregnancy was less than that in late normal pregnancy. The adrenaline excretion was the same in both groups.

The day time excretion of noradrenaline in the patients with toxemic pregnancy treated in hospital who were resting in bed also in the day, was 0.46 μg/hour, and that excreted by night 0.25 μg. This shows that the 24 hour rhythm was retained also in the patients resting, althoug the difference between the day and night excretion was diminished. The same observation has been made with regard to healthy people (v. EULER *et al.* 1955 b, SUNDIN 1956, ELMADJIAN *et al.* 1957, JANUSZEWICZ & WOCIAL 1960).

General discussion. Normally, even a comparatively slightly increased activity causes agitation of the sympathetic nervous system due to augmented urinary excretion of noradrenaline and adrenaline. Even standing up increases the excretion significantly (v. EULER *et al.* 1955 b). Hence it might be thought that also pregnancy — which markedly changes conditions in woman — might affect the sympathetic nervous system. The present investigation shows, however, that the urinary excretion of both noradrenaline and adrenaline remains completely within normal limits during pregnancy. Likewise, the day and night variation remains the same as in the non-pregnant women. Running practice causes a four to sixfold increase in the noradrenaline excretion in men, and a thirty fivefold augmentation is caused by strenuous work (KÄRKI 1956). During periods of stress, for instance such associated with major surgical operations of the abdomen or chest, or during matriculation examination, the noradrenaline excretion may increase twofold, and the adrenaline excretion fourfold (HAAME *et al.* 1957, PEKKARINEN *et al.* 1961). Increases in the noradrenaline and adrenaline excretion comparable with those mentioned were not observed during pregnancy.

Since the noradrenaline and adrenaline excretion was not increased during pregnancy even in the women occupied with day time work of a

strenuous kind there is a possibility that the homeostasis of pregnant women maintains mainly abundant reserves of blood and tissue fluid, and the sympathetic nervous system plays a minor role than in non pregnant women

As the excretion of noradrenaline and adrenaline remains normal also during toxemic pregnancy it is evident that these substances do not directly give rise to toxemia of pregnancy Yet certain signs have been observed which point to the fact that noradrenaline may be responsible in some degree for the arisal of toxemic symptoms For instance resting in bed decreases the excretion of noradrenaline in the urine and simultaneously alleviation of the toxemic symptoms is observed Reserpine, which is a useful drug for clinical administration in toxemic pregnancy, also decreases the noradrenaline excretion even in women occupied with light physical work

During infusion of noradrenaline the average rise in blood pressure is significantly greater in patients with toxemia of pregnancy than in those with normal pregnancy or in non pregnant women (RAAB *et al* 1956) and the decrease in the blood flow in the finger tips is greater in the first mentioned group than in the second (MENDLOWITZ *et al* 1961) The explanation of this sensitization may be that the estrogen (BROWN 1956) and progesterone (PEARLMAN 1957) production is increased during pregnancy and likewise the 17 OHCS content in the plasma (GEMZELL 1953 PEKKARINEN *et al* 1962) Furthermore the retention of sodium chloride in the tissues is increased during toxemic pregnancy (PARVIAINEN *et al* 1950 a FRIEDBERG 1959) In experimental tests (RAAB *et al* 1950 KURLAND & FRIEDBERG 1951) sensitization of the blood vessels has been achieved by pre treatment with cortisone desoxycorticosterone acetate and ACTH Simultaneous administration of adrenaline and sodium chloride over a long period raises the blood pressure significantly more than does administration of adrenaline alone (AHO *et al* 1961) On the other hand it is known that cortisone and ACTH pre treatment reduces the urinary excretion of noradrenaline (LUFT & v EULER 1952) This may explain why the noradrenaline excretion during pregnancy increases only slightly during physical exertion

It may be queried however, whether such a small increase in the excretion of noradrenaline is of significance with regard to development of toxemia of pregnancy in spite of the vascular system then reacting exceptionally sensitively to noradrenaline

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urine was clearly increased in only about 16 per cent of the 500 patients studied (v. Euler *et al.* 1954 b).

Moreover, only the free noradrenaline and adrenaline excretion in the urine was determined in the present investigation. These substances form a very small part of the metabolites excreted in the urine. According to previous investigations the amount of noradrenaline excreted in the urine of healthy persons is, after hydrolysis, on an average 152 to 178 per cent of that determined prior to hydrolysis (v. Euler & Orwén 1955, Pitkänen 1956) and, correspondingly, the average amount of adrenaline is 212 per cent (v. Euler & Orwén 1955). To what extent the catechol conjugation and metabolism is, in general, changed during pregnancy is not known. As the metabolism of hydrocortisone and the conjugation of 17-OHCS is retarded during pregnancy (Pekkarinen *et al.* 1962), it is possible that changes also in the metabolism of noradrenaline and adrenaline are liable to occur. For that reason no definite conclusions may be drawn regarding the role played by noradrenaline and adrenaline in the development of toxemia of pregnancy on the basis of the urinary excretion of these two substances in the free form.

The results of this study point, however, to the fact that the noradrenaline metabolism is somewhat changed during pregnancy and further studies on metabolization of catecholamines may elucidate also the etiology of toxemic pregnancy.

SUMMARY

In the present study an endeavour was made to evaluate the stress on the organism of pregnant women by determining the excretion of noradrenaline and adrenaline in urine in late normal and toxemic pregnancy special attention being paid to the effect of work and rest In addition the action of reserpine treatment during toxemic pregnancy on the excretion of noradrenaline and adrenaline was studied

Noradrenaline and adrenaline was biologically determined on the cat's blood pressure and the hen's rectal cecum according to PEKKARINEN's (1962) modification of v EULER's (1948) method using ascorbic acid for stabilization and sensitization of the hen's rectal cecum and to prevent auto-oxidation during alcohol acetone evaporation The recovery of noradrenaline was 80 per cent and that of adrenaline 78 per cent

The material comprises 233 pregnant women whose noradrenaline and adrenaline excretion in the urine was determined in late pregnancy, generally in the last trimester In 133 of these the pregnancy progressed normally and in 100 toxemia of pregnancy developed Fifty eight were resting in hospital and 27 were under reserpine treatment

In spite of the stress caused by pregnancy and the effect of toxemia the excretion of noradrenaline and adrenaline kept within normal ranges although slightly increased excretion was sometimes observed

In 105 women doing housework during normal pregnancy the excretion of noradrenaline was 19 7 ± 0 79 µg/24 hours and that of adrenaline 4 4 ± 0 27 µg/24 hours

In 38 women doing housework in late toxemic pregnancy the urinary excretion of noradrenaline 24 1 ± 2 2 µg/24 hours and that of adrenaline 4 5 ± 0 50 µg 24 hours was normal although the former was significantly greater than during normal pregnancy The noradrenaline excretion was increased in only one woman being 80 0 µg/24 hours

On study of the effect of physical work on the noradrenaline excretion in 14 women in late normal pregnancy normal values were observed average 24 0 ± 2 38 µg 24 hours yet this was significantly higher than in the women with normal pregnancy doing housework. The excretion of adrenaline was 4 9 ± 0 46 µg/24 hours ...

urine was clearly increased in only about 16 per cent of the 500 patients studied (v. Euler *et al.* 1954 b).

Moreover, only the free noradrenaline and adrenaline excretion in the urine was determined in the present investigation. These substances form a very small part of the metabolites excreted in the urine. According to previous investigations, the amount of noradrenaline excreted in the urine of healthy persons is, after hydrolysis, on an average 152 to 178 per cent of that determined prior to hydrolysis (v. Euler & Orwén 1955, Pitkänen 1956) and, correspondingly, the average amount of adrenaline is 212 per cent (v. Euler & Orwén 1955). To what extent the catechol conjugation and metabolism is, in general, changed during pregnancy is not known. As the metabolism of hydrocortisone and the conjugation of 17-OHCS is retarded during pregnancy (Pekkarinen *et al.* 1962), it is possible that changes also in the metabolism of noradrenaline and adrenaline are liable to occur. For that reason no definite conclusions may be drawn regarding the role played by noradrenaline and adrenaline in the development of toxemia of pregnancy on the basis of the urinary excretion of these two substances in the free form.

The results of this study point, however, to the fact that the noradrenaline metabolism is somewhat changed during pregnancy, and further studies on metabolization of catecholamines may elucidate also the etiology of toxemic pregnancy.

24 hours and the 24 hour urine volume remained unchanged (1260 and 1330)

The *diurnal rhythm* of noradrenaline and adrenaline excretion was normal in late normal and toxemic pregnancy. The average day time excretion of noradrenaline in late normal pregnancy was 14 0 ± 1 43 μg/12 hours and that by night 5 9 ± 0 38 μg/12 hours and the adrenaline excretion 3 5 ± 0 60 μg/12 hours and 2 0 ± 0 32 μg/12 hours respectively, in a group of 26 women. In late toxemic pregnancy the day time excretion of noradrenaline was 13 6 ± 3 10 μg/12 hours and that by night 5 1 ± 1 13 μg/12 hours and correspondingly, the excretion of adrenaline 4 1 ± 0 59 μg/12 hours and 1 6 ± 0 30 μg/12 hours in a group of 13 patients.

with toxemia of pregnancy doing physical work the value for noradrenaline excretion was the highest noted in the present study.

In 14 patients *resting in hospital* during normal pregnancy due to premature rupture of the fetal membranes or to prolonged pregnancy the noradrenaline excretion (13.7 ± 1.18 μg/24 hours) was significantly lower than in the women doing housework or physical work as well as in the non-pregnant women. The excretion of adrenaline (5.1 ± 1.00 μg/24 hours) was almost the same as that in the women occupied with housework or physical work. *The mean ratio of adrenaline to total noradrenaline* and adrenaline was 25.6 per cent in the women resting in hospital during normal pregnancy, 17.6 per cent in those doing housework and 18.2 per cent in those occupied with physical work. Four days rest in hospital did not markedly affect the excretion of noradrenaline (11.9 μg/24 hours on the first day and 10.7 μg/24 hours on the fourth). The adrenaline excretion increased in the first three days from 5.4 μg/24 hours to 10.4 μg/24 hours, but the increase was not significant.

The average noradrenaline excretion in 24 women resting in bed due to toxemia of late pregnancy (15.5 ± 1.38 μg/24 hours) was significantly less than that in the women with toxemia of pregnancy doing housework or physical work. The excretion of noradrenaline was 5.3 ± 0.64 μg/24 hours and the mean ratio of adrenaline to total noradrenaline and adrenaline 26.5 per cent was high.

The symptoms of toxemic pregnancy diminished during four days rest in hospital: the blood pressure decreased from 173/115 mmHg to 143/102 mmHg, the proteinuria from 0.8 to 0.5 g/24 hours and the 24-hour urine volume remained unchanged (955 ml and 1180 ml). At the same time the noradrenaline excretion changed from 14.9 μg/24 hours to 19.4 μg/24 hours.

In women given *reserpine treatment* for toxemic pregnancy occupied with housework of physical work the noradrenaline excretion was 10.4 ± 1.30 μg/24 hours and that in women resting in hospital 13.6 ± 2.40 μg/24 hours. Thus reserpine diminished the excretion *in women with toxemic* pregnancy doing housework or physical work but did not decrease significantly the excretion in patients resting in bed whose noradrenaline excretion was initially lower than normal. The adrenaline excretion was 4.2 ± 0.53 μg/24 hours and the mean ratio of adrenaline 25.1 per cent of the total amount of noradrenaline and adrenaline.

Reserpine reduced the noradrenaline excretion in patients with toxemic pregnancy resting in bed in hospital to 15.1 μg/24 *hours on* the first day and to 8.7 μg/24 hours on the fourth. At the same time the adrenaline excretion increased from 5.4 μg to 9.0 μg/24 hours. The blood pressure fell from 171/112 to 150/101 mmHg and the proteinuria from 1.6 to 1.3 g.

24 hours and the 24 hour urine volume remained unchanged (1260 and 1330)

The *diurnal rhythm* of noradrenaline and adrenaline excretion was normal in late normal and toxemic pregnancy The average day time excretion of noradrenaline in late normal pregnancy was 14.0 ± 1.43 μg/12 hours and that by night 5.9 ± 0.38 μg/12 hours and the adrenaline excretion 3.5 ± 0.60 μg/12 hours and 2.0 ± 0.32 μg/12 hours respectively, in a group of 26 women In late toxemic pregnancy the day time excretion of noradrenaline was 13.6 ± 3.10 μg/12 hours and that by night 5.1 ± 1.13 μg/12 hours and correspondingly, the excretion of adrenaline 4.1 ± 0.59 μg/12 hours and 1.6 ± 0.30 μg/12 hours in a group of 13 patients.

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Hemodynamic Effects of Hydralazine

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III ÅBLAD B G JOHNSSON & M HENNING The effects of hydralazine administered into the brachial artery on adrenergic vasoconstrictor stimuli in the hand *Acta pharmacol et toxicol* 1962 *19* 165—180

IV ÅBLAD, B & G JOHNSSON *Comparative effects of intra arterially* administered hydralazine and sodium nitrite on *blood flow* and volume of forearm *Acta pharmacol et toxicol* 1963 *20* 1—15

These *communications* will be referred to as I—IV

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INTRODUCTION

In 1950 GROSS, DRUEY and MEIER reported that a group of compounds containing a phtalazine ring together with a hydrazine side chain exhibited hypotensive activity. Of these compounds 1-hydrazinophthalazine — also known as hydralazine — was subsequently introduced for therapeutic use under the trade name of apresoline. It has since been extensively used in the treatment of hypertension. More than 1000 communications were published in the years 1950—1961 dealing with various aspects of the pharmacology of the drug.

The acute hemodynamic effects elicited by hydralazine are well established. In man intravenously administered hydralazine in a dose of 0.2—0.5 mg/kg produces a slowly developing moderate reduction of arterial blood pressure lasting several hours. This effect must be due to a decrease of the peripheral vascular resistance, since the cardiac output as a rule is increased, both by increased stroke volume and increased heart rate (WILKINSON *et al.* 1952, FREIS *et al.* 1953, STEIN & HECHT 1955).

Several studies have been made on anesthetized animals concerning the mechanism of vasodilator action of the drug. The results of these studies have varied and there are divergent opinions as to the question whether the site of action for the vasodilator effects is located in the central nervous system or in the peripheral vascular bed. In a recent survey of the hemodynamic effects elicited by hydralazine in man FREIS (1961) states that "despite considerable investigation the mechanism by which hydralazine produces these hemodynamic changes has not been clarified".

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THE PRESENT INVESTIGATION

The main purpose of the present investigation was to analyse the therapeutically utilized vasodilator action of hydralazine It was considered essential to study this problem in unanesthetized human beings rather than in anesthetized animals Previous investigations on animals aiming at determining to what extent the vasodilator action of the drug was dependent upon a central point of attack had furnished contradictory results a feasible explanation could be that the relative significance of this effect varied according to the selection of anesthetic and/or depth of anesthesia Also the administration of hydralazine in large doses produced only moderate circulatory effects which developed slowly and remained a long time Accurate analyses of the action of such drugs are more difficult on anesthetized traumatized animals where the general state of the circulation may change during the course of the experiment Analytical studies on *Homo* are also necessary to establish that interpretations based on studies in animals are relevant to the action of therapeutic amounts of a drug in man

From what was mentioned in the introduction it was evident that the first problem to be studied was the following Is the site of action for the vasodilator effect of hydralazine predominantly located in the central nervous system or in the peripheral vascular bed? This problem was studied by recording the vascular responses to intravenously administered hydralazine in the upper part of the forearm or the hand using a technique which made it possible to differentiate the relative importance of the postulated central and peripheral points of action It was found (I) that the peripheral point of action was of predominant importance for the decrease of peripheral vascular resistance produced by hydralazine Furthermore the results indicated that the sympathetic vasoconstrictor fibre discharge was increased after administration of the drug and thus contradicted the opinion that the vasodilator action of the drug was due to a central inhibition of sympathetic vasoconstrictor tone — It was further found (II) that a small dose of hydralazine administered intra arterially produced a longlasting decrease of the vascular resistance in the forearm and the hand This result lent further support to the view that the vasodilator effect of the drug was predominantly due to its peripheral site of action This question will be further discussed below in sections 1 and 2

These results raised a second question Is the vasodilator effect of hydralazine due to a specific peripheral antagonism to sympathetic vasoconstrictor tone or to a direct action on vascular smooth muscle? Therefore a study was

made of how intra arterially administered hydralazine influenced the vaso constrictor action in the hand caused by noradrenaline or by a reflex sympathetic stimulus (III) Hydralazine was compared to vasodilator agents producing a specific peripheral antagonism to sympathetic vasoconstrictor tone It was found that hydralazine differed from these drugs in one important respect a small dose of hydralazine produced a distinct vasodilatation but did not markedly weaken the vasoconstrictor stimuli This result indicates that the vasodilator effect of the drug was due to a direct action on the vascular smooth muscle Further comments on this question will be made in section 3

These studies gave information on how hydralazine influenced the vessels which determine the resistance to blood flow (mainly arterioles and smaller arteries) The next question was How does hydralazine influence the vessels which determine the peripheral vascular capacity of blood (mainly venules and veins)? This was studied by comparing the effects of intra arterially administered hydralazine and sodium nitrite on functionally differentiated series coupled vascular sections in the upper part of the forearm (IV) In comparison with sodium nitrite hydralazine produced a more pronounced relaxation of the vessels which determine vascular resistance but a smaller relaxation of the vessels which determine vascular blood capacity This question will be further dealt with in section 4

These findings raised a new question Are the differentiated vascular response patterns for hydralazine and sodium nitrite in the forearm representative of their action on the general systemic circulation? Experiments elucidating this question are reported in section 5 It was found that hydralazine produced a more pronounced decrease of total peripheral vascular resistance than an equihypotensive dose of sodium nitrite The results further indicated that hydralazine evoked a smaller increase of total peripheral vascular blood capacity than an equihypotensive dose of sodium nitrite Furthermore the general hemodynamic effects of intravenously administered hydralazine and sodium nitrite were characterized by differences that could be ascribed to differentiated vascular response patterns in the general systemic circulation of the same type as found by a more direct approach in the forearm

It therefore seems probable that hydralazine s site of action in the peripheral vascular bed is the primary *determinant* of its hemodynamic effects in man This view appears to be relevant also to its circulatory effects in hypertensive patients as will be discussed in section 6

SECTION 1

Is the predominant site of action for the vasodilator effect of hydralazine located in the central nervous system or in the peripheral vascular bed?

a *Current opinions* As mentioned in the introduction it is generally agreed that the hypotensive action of hydralazine is due to a decrease of the peripheral vascular resistance but there are widely different opinions as to what extent this effect is caused by an inhibition of the sympathetic vasoconstrictor nerve fibre discharge from the central nervous system

There seems to be no doubt that hydralazine has a point of action in the peripheral vascular bed In isolated vascular preparations studied *in vitro* the drug weakens the effect of vasoconstrictor substances (see section 3 h below) *In vivo* hydralazine weakens the hypertensive effect of noradrenaline and adrenaline (Gross *et al* 1950, Moyer *et al* 1951, Bein *et al* 1953 Kirpekar & Lewis 1957) However the antagonism to sympathomimetic pressor agents has been found to be relatively weak (Craver *et al* 1951 Walker *et al* 1951, Moyer *et al* 1953) and this seems to be one of the reasons why some authors have been uncertain if the drug's peripheral site of action is of any greater importance *in vivo* (e g Craver *et al* 1951)

The opinion that hydralazine has a central site of action of importance for its vasodilator effect has been based on studies by Craver *et al* (1951) Bein *et al* (1953) and Tangri & Bhargava (1961) In these investigations the arterial blood pressure was recorded and the results obtained with various experimental procedures were interpreted as evidence of a central site of action (see below) By the use of essentially similar techniques however other investigators were unable to reveal the existence of a central point of action (Grimson *et al* 1952 Schmitt & Giquel 1956 Kirpekar & Lewis 1957) Bein *et al* (1953) reported that hydralazine antagonized the reflexogenic pressor response after occlusion of both carotid arteries or after stimulation of the central stump of the sciatic or vagal nerve to a greater degree than the pressor response induced by intravenously injected noradrenaline or by stimulation of the peripheral stump of the splanchnic nerve This finding was considered to indicate the existence of a central point of action since the drug was found to be devoid of ganglionic blocking properties However Kirpekar & Lewis (1957) could not find that hydralazine produced such a differentiated antagonism to these pressor stimuli According to Tangri & Bhargava (1961) hydralazine administered in the 4th cerebral ventricle produced hypotension without weakening the pressor action of intravenously injected noradrenaline Schmitt & Giquel (1956) however found that the hypo

tensive action of hydralazine administered in this manner was accompanied by a weakening of the hypertensive effect of intravenously injected adrenaline and concluded that the hypotensive effect of the drug even when administered in this manner was due to a peripheral site of action In experiments on the cross circulated head of a dog TANGRI & BHARGAVA (1961) made observations indicating a central inhibition of sympathetic discharge but with this technique GRIMSON *et al* (1952) could find no evidence of a central site of action CRAVER *et al* (1951) found that hydralazine was devoid of hypotensive action in the spinal' cat or dog and this observation was considered to indicate that the hypotensive effect of the drug was due predominantly to a central site of action

The contradictory results obtained in these blood pressure studies clearly indicated that the question had to be settled by an experimental approach which allowed a more differentiated study of the factors determining the peripheral vascular resistance In anesthetized animals more conclusive information can often be obtained by studying the changes of the vascular resistance in a peripheral cross circulated tissue with intact sympathetic innervation The action of hydralazine was analysed with such a technique by PAGE & MCCUBBIN (1953) Their results indicated that the vasodilator effect of the drug was due to its peripheral site of action This study will be further discussed below

b *Experimental* In human beings there seems to be no technique generally suitable for a more direct differentiation of the relative importance of a possible central and peripheral site of action for the vascular response of a drug However it is sometimes possible to make such a study even *in man* For example BARCROFT *et al* (1951) have devised a technique suitable for analysing drugs which leave the blood rapidly are not redistributed too fast and have an action of fairly long duration By completely occluding the circulation through one arm during and for some minutes after the intravenous injection of such a drug it is possible to prevent access of the drug to this arm When the circulation is re opened to the arm there will at first occur a local reactive hyperemia lasting for some minutes Thereafter, any difference between the blood flow in the two arms not manifest prior to the injection will be due to the fact that one arm contains more of the administered drug than the other

It was considered possible to differentiate the proposed central and peripheral sites of action of hydralazine by this technique Studies in animals (1) indicated that the amount of free drug in the blood decreased rapidly after intravenous injection this finding has recently been confirmed in a more detailed study by SCHULERT (1961)

An intravenous injection of 0 2 mg hydralazine per kg body weight produced a small decrease of the mean arterial pressure an increase of the heart rate

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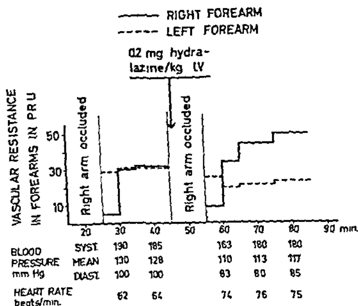


Figure 1. Effects of hydralazine (0.2 mg/kg i.v.) on blood pressure, heart rate and vascular resistance in upper part of the forearms in male patient with benign hypertension. Supine position. Blood pressure measured by means of the RIVA ROCCI auscultatory method; diastolic pressure determined when sounds suddenly began to fade. Mean arterial blood pressure calculated by adding 1/3 of pulse pressure to diastolic pressure. The approximate vascular resistance in the forearms was calculated by dividing mean arterial blood pressure (mm Hg) by blood flow in the forearms (ml/min/100 ml tissue). The term peripheral resistance units, P.R.U., corresponds to the PRU_{100} of GREEN (1948, p. 243). Venous blood samples for determination of noradrenaline and adrenaline were taken at the 40th and 70th minutes (see text). — Notice that the initial occlusion of the circulation to the right arm for 10 minutes was followed by a local reactive hyperemia, which had disappeared completely 5 minutes after the end of the occlusion. When hydralazine was injected i.v., it evoked a prolonged decrease of the vascular resistance in the left forearm but a prolonged increase of the resistance in the right forearm, whose circulation was occluded immediately before and for 10 minutes after the injection of the drug.

and a small decrease of the vascular resistance in the forearm with intact circulation during and after the administration of the drug (I) However, in the other forearm which had been excluded from the circulation during and for ten minutes after the injection of the drug there occurred a pronounced and persistent increase of the vascular resistance A similar differentiated response occurred in the hands as could be judged from a pronounced difference in the colour and temperature of the skin this difference persisted for several hours

The hydralazine related drug dihydralazine whose action from a qualitative point of view appears to be similar to that of hydralazine (animal experiments GROSS *et al* 1950 WALKER *et al* 1951 SCHMITT & GICQUEL 1956 KIRPEKAR & LEWIS 1957, clinical investigations ESSFLIER *et al* 1953 PETERS 1954) — elicited a similar differentiated vascular response pattern in the forearms and hands (I) Dihydralazine seemed to produce more pronounced circulatory effects than the same dose of hydralazine

The results just reported were obtained in normotensive human beings In 3 recent experiments on hypertensive patients the effects of hydralazine on the forearm circulation were studied with the same experimental procedure (JOHNSSON HENNING HÄGGENDAL ÅBLAD *unpublished observations*) In these patients hydralazine produced a differentiated vascular response in the two forearms similar to that obtained in normotensive subjects A typical experiment is shown in fig 1

The finding that intravenously administered hydralazine produced a vasodilatation only in that arm whose circulation was not occluded during and after the administration of the drug indicated that the peripheral localization of the drug was of predominant importance for its vasodilator action The persistent differentiated vascular response in the hands further indicated that the drug was firmly bound to peripheral receptors The pronounced increase of the vascular resistance in the arm excluded from circulation during the distribution of the drug to the tissues did not appear if the median ulnar and radial nerves were blocked by a local anesthetic agent at the elbow (I) This suggested that the increased vascular resistance was predominantly due to an increased vasoconstrictor fibre discharge to the hand

In the above study on hypertensive patients determinations were made of the concentrations of noradrenaline and adrenaline in arterial or venous blood before as well as 25—40 minutes after the administration of hydralazine (determinations performed according to HÄGGENDAL (1963)) In all 3 experiments the concentration of the two catecholamines were within normal levels and no certain increase in the concentrations occurred after the administration of hydralazine This observation indicated that in the dose given hydralazine did not release any significant amount of catecholamines from the adrenal medulla The finding was in line with the above reported plethys

SECTION 2

Regional vascular effects of intra arterially administered hydralazine

As the peripheral site of action appeared to be of predominant importance for the vasodilator effect of intravenously administered hydralazine, it was considered to be of interest to investigate this action by a more direct approach This was done by studying how hydralazine administered in a brachial artery influenced the vascular resistance in the hand or in the upper part of the forearm (II) The drug was given in a low dosage and it was presumed that its tissue concentration in the arm was within the range associated with systematic administration of therapeutic doses The results indicated that hydralazine evoked a decrease of the vascular resistance in the hand and fore arm on the side where it was given but no effects in the other arm The regional vasodilatation developed gradually and reached its maximum 12–20 minutes after the administration The effect was then fairly constant for about 1 hour and in some experiments it had not disappeared completely even 4 hours after the administration The time course for this effect was very similar to the time course for the hypotensive effect of intravenously administered hydralazine These data gave further support to the view that the peripheral site of action was of predominant importance for the vasodilator effect of hydralazine and that the drug (or the active metabolite) was relatively firmly attached to its receptors in the vascular bed

It would be of interest to know if intra arterial administration of small doses of hydralazine to other tissues would produce a response similar to that in the forearm and the hand The effects of intra arterial hydralazine on the kidney blood flow was studied in two investigations on dogs (PAGE & MCCUBBIN 1953 MOYER 1959) but the dose was relatively large In the above discussed study by PAGE & MCCUBBIN (1953) it was found that subsequent to intra arterial administration of 5 mg hydralazine directly to the cross circulated kidney there occurred an initial increase of the vascular resistance in the kidney followed by a slowly developing vasodilation of long duration MOYER (1959) found that hydralazine administered into a renal artery pro duced a decreased plasma flow in the kidney to which it was given but an increased plasma flow in the other kidney The author does not specify the dose but it was evidently relatively large since [illegible]
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mographic observations which indicated that the greater part of the vaso constrictor effect of hydralazine in the "occluded" hand was due to increased discharge in the vasoconstrictor nerves

c *Discussion* The reported results indicated that hydralazine elicited an increased vasoconstrictor fibre discharge to the arm which would tend to counteract the vasodilator effect induced by the drug's peripheral site of action This interpretation coincides with results reported by STUNKARD *et al* (1954) These authors studied the peripheral circulatory effects of intravenously administered hydralazine on patients in whom one or two limbs were devoid of sympathetic innervation Hydralazine was found to produce an increased blood flow in sympathectomized limbs but an unchanged or decreased blood flow in normally innervated limbs — The interpretation suggested above is also relevant to the results obtained by PAGE & McCUBBIN (1953) on the cross circulated dog kidney with intact innervation When hydralazine was given to the "donor" animal whose circulation was connected to the kidney under study, there occurred a decrease of the vascular resistance in this kidney If hydralazine was given to the body of the "receptor" animal it did not reach the cross circulated kidney but could influence its vessels via the nervous pathways When hydralazine was so administered it reduced the systematic arterial pressure of the "receptor" animal and the vascular resistance of the cross circulated kidney was increased The latter effect was apparently due to increased sympathetic vasoconstrictor fibre discharge to the kidney This vasoconstrictor response did not appear if the aortic depressor and carotid sinus nerves of the "recipient" dog were cut before hydralazine was given to it This may indicate that the increased vasoconstrictor fibre discharge induced by hydralazine was a reflex response due to decreased pressure in the aortic and carotid sinuses

It should be noted that the positive chronotropic effect on the heart produced by hydralazine also appears to be due to an increased sympathetic discharge since it has been found to be largely abolished by cutting the cardiac sympathetic nerves (GRIMSON 1952) and greatly reduced after administration of hexamethonium in dogs (MOYER *et al* 1953) and man (STEIN & HECHT 1955) It is possible that this response is also a reflex adjustment secondary to the peripheral vasodilatation induced by the drug (see further section 4f)

d *Conclusions* Hydralazine has a site of action in the peripheral vascular bed which is of predominant importance for its vasodilator action in man The drug appears to increase the discharge in the vasoconstrictor and cardio accelerans nerves This response may be a reflex adjustment to the vasodilator effect The increased vasoconstrictor fibre discharge after administration of hydralazine contradicts the view that the vasodilator effect of the drug to any significant extent is due to a reduction of central vasomotor tone

produced by a "directly" acting vasodilator drug should be relatively weaker than that evoked by an adrenergic blocking agent if the antagonistic response were related to the vasodilator effect of the drugs in a vascular region exhibiting myogenic tone

In view of the unspecificity of many vasodilator drugs and the complicated physiology of vascular smooth muscle it seems important that the experimental characterization of a vasodilator drug includes studies with therapeutic doses of the drug otherwise the results may be irrelevant to the therapeutic action of the drug

c *Current opinions on the mechanism for the peripheral vasodilator action of hydralazine* Some authors consider the peripheral vasodilator effect of hydralazine to be due to a weak adrenergic blockade This view is based upon the finding that intravenously administered hydralazine weakens the hypertensive effect of noradrenaline and adrenaline (e g GROSS *et al* 1950 MOYER *et al* 1951) Another opinion is that the vasodilator action of the drug is due to a "direct" action on vascular smooth muscle This view is in part based on *in vitro* studies which have shown that hydralazine decreases the spontaneous vascular tone and exerts a non specific antagonism to various vasoconstrictor agents (see further below under *h*) STUNKARD *et al* (1954) furnished evidence that the vasodilator action of therapeutic doses of hydralazine in normotensive and hypertensive human beings is due to a "direct" action on vascular smooth muscle The evidence was founded on two different observations Firstly intravenous administration of hydralazine in a low hypotensive dose did not change the hypertensive effect of a reflex sympathetic stimulus (cold test) or of intravenously infused noradrenaline Secondly when hydralazine was administered intravenously to patients in which one or two limbs were devoid of sympathetic innervation it was found that the drug increased the blood flow in the "sympathectomized" limbs but decreased or did not change the flow in normally innervated limbs

d *Effects of intra arterially administered hydralazine on vasoconstrictor stimuli in the hand* The results of STUNKARD *et al* appear convincing but nevertheless it seemed essential to study the question also by a more direct approach An attempt was therefore made to analyze the vasodilator action of intra arterially administered hydralazine in the hand (III) This was done by studying the interaction of hydralazine with the vasoconstrictor effects of a reflex sympathetic stimulus (cold test) and of intra arterially or intravenously infused noradrenaline in the vessels controlling the regional resistance in the hand The cold test produced an almost immediate vasoconstrictor response in the hand this immediate response was considered to be due to an increased discharge in the vasoconstrictor fibres to the hand

SECTION 3

Mechanism for the peripheral vasodilator action of hydralazine

a *Some physiological aspects on vascular tone* Vascular smooth muscle, like most other smooth muscle, can exhibit a spontaneous myogenic tone Under basal' conditions *in vivo* the vessels which control the resistance to blood flow appear to exhibit 'myogenic' tone, but its extent varies markedly from one region to another Physiologically occurring vasoconstrictor substances should be regarded more as modulators of this 'myogenic tone than as ultimate initiators of vascular tone Presently available evidence seems to indicate that under physiologic conditions the most important vasoconstrictor adjustor of myogenic vascular tone is noradrenaline released from the sympathetic vasoconstrictor nerve endings — For further details on the physiology of vascular smooth muscle see FURCHGOTT (1955) FOLKOW (1956) and FOLKOW (1962 a)

b *Classification of peripherally acting vasodilator agents* In view of the discussion above peripherally acting vasodilator drugs may be classified functionally into 3 groups

1 Agents producing peripheral adrenergic nerve blockade e g guanethidine bretylium and reserpine, the vasodilator action of which presumably is mainly due to a diminished release of noradrenaline from the sympathetic vasoconstrictor nerve endings (see e g review by GREEN 1962)

2 Adrenergic blocking agents i e agents whose vasodilator action is due to blockade at the adrenergic α receptor (see e g reviews by NICKERSON 1949 GREEN 1958 NICKERSON 1959) All such agents known at present are characterized only by a relative specificity, and at higher dosage they may all interfere with receptors in vascular smooth muscle other than the adrenergic α receptor (cf FURCHGOTT 1955 NICKERSON 1959)

3 Agents with a direct action on vascular smooth muscle e g substances like nitrites and other general smooth muscle relaxants the vasodilator effect of which is primarily due to a depression of the myogenic tone of vascular smooth muscle In view of the above discussed physiological considerations one would expect these drugs to weaken to a certain degree the vasoconstrictor effect of both the adrenergic transmittor and agents acting on receptors other than the adrenergic α receptor However this antagonism to noradrenaline

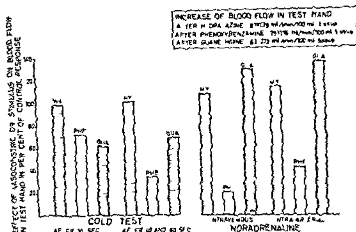


Figure 2 Effects of intra arterially administered hydralazine (0 30—0 65 mg), phenoxybenzamine (0 02—0 10 mg) and guanethidine (0 5—2 0 mg) on hand blood flow (inset) on vasoconstrictor effect of a cold test (bars to the left) and on vasoconstrictor effect of noradrenaline (bars to the right) The cold test was applied for 70 seconds Its vasoconstrictor effect in the hand was estimated by determining the difference between the blood flow before the test and 10 40 and 60 seconds after its application — Noradrenaline bitartrate was infused intravenously [illegible]

[illegible]

col [illegible] noradrenaline the vasoconstrictor response in the drug treated test hand was related to the simultaneously occurring vasoconstrictor response in the untreated contralateral hand (control hand) for intra arterially infused noradrenaline the vasoconstrictor response in the drug treated test hand was [illegible] to the vasoconstr[illegible]

noradre[illegible]

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the vas[illegible] conception of how one vasodilator drug influenced the vasoconstrictor stimuli in comparison to the two others The average resting blood flow in the two hands was [illegible] the phenoxybenzamine and guanethidine [illegible] and the average blood flow in the cont[illegible] administration of vasodilator drug to th[illegible] The average vasoconstrictor control responses were also quantitatively similar in the three series For further details on these results see communication III and HENNING et al (1963)

A great part of the blood vessels in the hand are engaged in temperature regulation and their resistance to a great extent is determined by the sympathetic vasoconstrictor tone. However, there is evidence that part of the vascular resistance in the hand is not maintained by vasoconstrictor nerve activity (for references see III) and it seems reasonable that this non-nervous component is at least to some extent due to "myogenic" tone. The experiments were performed in a fairly high room temperature with the object of having a relatively low "basal" level of vasoconstrictor fibre discharge to the hand.

When hydralazine was administered intra-arterially in a low dose considered to establish a "therapeutic" concentration of the drug in the hand (see section 2) it produced a distinct decrease of the vascular resistance in the hand but it did not markedly weaken the vasoconstrictor stimuli. This result was considered to indicate that the vasodilator effect of hydralazine was mainly due to a "direct" action on vascular smooth muscle. However, the relevance of this interpretation appeared somewhat uncertain since it was for several reasons difficult to estimate the degree of antagonism and its significance. Thus, for one thing, hydralazine changed the "basal" level of vascular resistance and it is hard to evaluate the degree to which such a change of the "basal" level of vascular resistance *per se* may influence the magnitude of vasoconstrictor responses in this type of experiments.

As it appeared necessary to make complementary studies of the question it was investigated whether the effects of hydralazine on the vasoconstrictor stimuli could be distinguished from the effects on these stimuli of peripherally acting agents assumed to produce vasodilatation by release of vasoconstrictor nerve tone (HENNING, JOHNSSON & ÅBLAD 1963). Two such agents were studied. One was phenoxybenzamine, whose vasodilator action is considered to be primarily due to α-receptor blockade (GREEN 1958), the other was guanethidine, which is considered to act through peripheral blockade of the adrenergic nerve transmission (review by GREEN 1962). When administered in low dosage into the brachial artery, both these agents produced a long-lasting decrease of the vascular resistance in the hand, but no obvious systemic effects. Phenoxybenzamine and guanethidine were given intra-arterially in a dose which produced about the same mean increase of the hand blood flow as hydralazine. This probably implied that all three drugs evoked about the same mean decrease of the vascular resistance in the hand, since the average "basal" hand blood flow recorded before the drugs were given was about the same in all three series. The average control responses to the vasoconstrictor stimuli were also similar in the three series.

Phenoxybenzamine, guanethidine and hydralazine produced different response patterns to the vasoconstrictor stimuli (Fig. 2). The effect of the cold test was antagonized to a higher extent by phenoxybenzamine and guanethidine than by hydralazine. The effect of noradrenaline was markedly

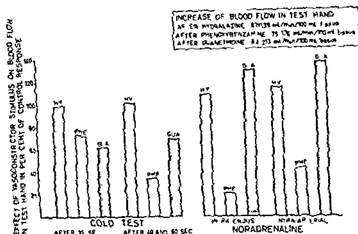


Figure 2 r· . . benzaminc . . . on vasoco .. (bars to the left) and on vasoconstrictor effect of noradrenaline (bars to the right) The cold test was applied for 70 seconds Its vasoconstrictor effect in the hand was estimated by determining the difference between the blood flow before the test and 10, 40 and 60 seconds after its application — Noradrenaline bitartrate was infused intraveno sly (10 ..

The effects of the vasoconstrictor stimuli in the hand treated with a vasod lator drug (test hand) are expressed in per cent of the control response This means that for the cold test and intravenously infused noradrenaline the vasoconstrictor response in the drug treated test hand was related to the simultaneously occurring vasoconstrictor response in the untreated contralateral hand (control hand) for intra arterially infused noradrenaline the vasoconstrictor response in the drug treated test hand was related to the vasoconstrictor response produced by the same dose of intra arterially infused noradrenaline in the test hand before it was treated with a vasodilator drug Each val ie given in the figure represents the mean result of 3—10 experiments The values give no information of the absolute degree of antagon ... .

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These results indicate that the vasodilator action of phenoxybenzamine was predominantly due to blockade of the adrenergic α receptor and that the vasodilator effect of guanethidine was predominantly due to blockade of the adrenergic nerve transmission The different results obtained with hydralazine indicate that its vasodilator action was due to depression of the myogenic *vascular tone including* only a relatively weak antagonism to sympathetic vasoconstrictor tone The finding was considered to support the view that the vasodilator action of hydralazine is mainly due to a direct action on vascular smooth muscle

In another series of experiments a higher dose of hydralazine was infused intra arterially (III) and its influence on the vasoconstrictor action of nor adrenaline and the cold test was investigated in the same way as in the above mentioned series The higher dose of hydralazine produced a more pronounced vasodilatation in the hand than the lower dose It was further found to evoke a pronounced antagonism to the vasoconstrictor action of noradrenaline whereas the effect of the cold test was possibly antagonized to a lesser extent

e *Effects of intravenously administered hydralazine on hypertensive stimuli* If the vascular response to hydralazine in the hands is representative of its effects on the peripheral circulation in general then the intravenous administration of a lower dose of the drug should produce a hypotensive effect without weakening adrenergic pressor stimuli to any great extent while a higher dose should evoke a definite weakening of such pressor stimuli This seems to be the case in man to judge from the studies of FREIS & LINNERTY (1950) GRIMSON et al (1950) FREIS et al (1951) and STUNKARD et al (1954) Studies in animals also point in the same direction (e g WALKER et al 1951) A higher dose of hydralazine may be a pronounced antagonist of weaker adrenergic stimuli to judge from the finding that the drug sometimes completely inhibits the pressor response to noradrenaline and evokes adrenaline reversal (e g MOYER et al 1951) The fact that this type of response is not a constant finding indicates that it is probably not due to direct blockade of the adrenergic α receptor but rather an expression of non specific antagonism to vasoconstrictor stimuli secondary to the depression of the myogenic vascular tone In agreement with such a view is the fact that in animals hydralazine has been proved sometimes also to weaken the hypertensive effect of pitressin (BEIN et al 1952) and angiotensin (MEIER et al 1957 a)

f *Effects of hydralazine on parallel coupled resistance circuits in man* The effects of systemically administered hydralazine on the vascular resistance in

various "parallel coupled" circuits in man have been studied extensively. The results are in agreement with the view that the "direct" action of the drug on vascular smooth muscle is of predominant importance for its vasodilator effect. Thus it has been found that the hypotensive effect of the drug is often accompanied by an increase of the blood flow in the kidneys, the brain and in the coronary circulation (for references see III). As the vasoconstrictor nerves are considered to be of little importance in controlling the blood flow in these regions under "resting" conditions (see e.g. FOLKOW 1956) the pronounced decrease of the regional resistance produced by hydralazine can probably be explained only by a reduction of the "myogenic" tone of the vascular smooth muscle in these organs. Further evidence in support of this view has been provided by STEIN & HECHT (1955). These authors found that hydralazine often evoked a pronounced increase of the renal blood flow even in patients pretreated with hexamethonium in a dose that largely abolished the stimulating effect of hydralazine on the heart.

Studies in man of the circulatory effects of intravenously administered hydralazine in other regions indicate that the drug evokes a pronounced vasodilatation in the liver (FREIS *et al.* 1953), while it seems to have a weaker vasodilator effect in the extremities. Thus it does not considerably change the blood flow in the forearm or in the leg (FREIS *et al.* 1953, STUNKARD *et al.* 1954, communication I) and it does not often increase the skin temperature in the extremities (FREIS & FINNERTY 1950, WILKINSON *et al.* 1952, STUNKARD *et al.* 1954). At higher room temperature, however, it seems to increase skin temperature in the hand (FREIS & FINNERTY 1950).

g. *Factors of possible importance for the differentiated response on "parallel coupled" resistance vessels.* The above discussed findings indicate that hydralazine does not produce a uniform decrease of the peripheral resistance. It should be recalled (see section 1 c) that the effect of hydralazine on the regional resistance seems to represent the result of two opposing actions, one vasodilator due to the peripheral site of action and one vasoconstrictor, due to the increased activity in the vasoconstrictor nerves. The magnitude of each of these two opposing factors may vary from one tissue to another and such a circumstance may explain the differentiated vascular response pattern produced by the drug. The vasodilator component may vary in different regions depending on different sensitivity of the resistance vessels and also depending on uneven distribution of the drug to the resistance vessels in various "parallel coupled" circuits. There are reasons to believe that the second possibility is of some importance at least as regards the effects of intravenously administered hydralazine. The results reported in communications I and II indicated that hydralazine left the blood rapidly and that it was firmly bound to its receptors in the vascular wall. Thus a relatively large

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amount of hydralazine might be distributed to tissues with high blood flow, resulting in a relatively pronounced and longlasting vasodilatation in such tissues. To test this hypothesis some experiments were performed in which the blood flow in both forearms or both hands was recorded simultaneously by venous occlusion plethysmography (ÅBLAD unpublished). When hydralazine was injected intravenously, the blood flow was kept higher on one side than on the other by muscle work or through local heating (high temperature of the water in the plethysmograph). Five or ten minutes after the injection the muscle work was stopped or the water temperature in both plethysmographs was equilibrated. Hydralazine was found to produce a considerably greater increase of the blood flow in the arm with the highest blood flow at the moment of the injection than in the other arm. The difference persisted for more than one hour. This persistent difference could not be found in experiments of the same type when isotonic saline was injected instead of hydralazine. The differentiated response produced by hydralazine was probably due to the fact that more drug was distributed to the vessels in the arm with the higher blood flow at the moment of the injection and this uneven distribution was evidently persistent for more than one hour. It is possible that these experiments have revealed at least a contributory cause of the fact that the vasodilator effect of hydralazine often is pronounced in highly vascularized organs like the kidneys and the liver.

b. *Vascular effects of hydralazine in vitro.* Hydralazine has been found to relax vascular smooth muscle also in *in vitro* preparations. According to BEIN *et al.* (1952) and TRIPOD & MEIER (1958) the drug dilated the coronary vessels in the isolated cat or rabbit heart and antagonized the vasoconstrictor effect of vasopressin and barium chloride in this preparation. The spontaneous tone of isolated horse carotid artery was decreased by hydralazine (KIRPEKAR & LEWIS 1958 *a*). The drug was found to reduce the vasoconstrictor effect of sympathomimetic amines, serotonin, angiotensin, histamine, acetylcholine, barium and potassium ions in isolated vessels suspended in an organ bath or in isolated perfused tissues (BEIN *et al.* 1953, SCHROEDER *et al.* 1955, KIRPEKAR & LEWIS 1957, MEIER *et al.* 1957 b, MEIER *et al.* 1958, TRIPOD & MEIER 1958). The results of these *in vitro* studies appear to be consistent with the view that the drug relaxes the vessels because of a "direct" action on vascular smooth muscle.

It was noticed by BEIN *et al.* (1953) that in the isolated hind leg of the rabbit hydralazine produced only a weakening of the effect of vasoconstrictor agents but never a complete inhibition and furthermore the drug exerted antagonistic action only at a narrow dose range. In these respects hydralazine was found to differ from other spasmolytic agents such as papaverine which exerted increasing antagonism to complete inhibition with increasing dose.

A similar result was recently reported by PERRY & CAMEL (1960) in a study on the influence of hydralazine on adrenaline induced contractions in strips of rabbit aorta. Hydralazine produced a moderate antagonism at a concentration between 5×10^{-7} and 10^{-6} but the antagonism was much less pronounced if the concentration of hydralazine was higher.

In an attempt to analyze the mechanism behind these interesting findings, a study was made of how hydralazine, papaverine and sodium nitrite influenced the vasoconstrictor action of noradrenaline on isolated bovine mesenteric artery under aerobic and anaerobic conditions (ÅBLAD unpublished). The results indicated that hydralazine (Fig. 3 A) weakened the vasoconstrictor action of noradrenaline under aerobic conditions. The antagonistic effect was most pronounced when the concentration of hydralazine was 10^{-6} and diminished at higher concentrations. In this respect the results were generally in agreement with those of BEIN et al. (1953) and PERRY & CAMEL (1960). Under anaerobic conditions, however, hydralazine did not antagonize noradrenaline, and at higher concentrations it was instead found to potentiate the vasoconstrictor response. — Similar tests with papaverine (Fig. 3 B) and sodium nitrite (Fig. 3 C) indicated that these two drugs antagonized the action of noradrenaline also in the absence of oxygen, and the antagonistic response was increased as the concentration of the spasmolytic agent was increased.

In some other experiments on the isolated bovine mesenteric artery, it was found that at a concentration of 10^{-5} hydralazine weakened the vasoconstrictor response to histamine and barium ions under aerobic conditions but potentiated the response under anaerobic conditions. Furthermore, it was found that at a concentration of 10^{-5} hydralazine exaggerated the spontaneous decrease of the "intrinsic" tone in this preparation under aerobic conditions but reduced the spontaneous decrease of tone under anaerobic conditions.

These results seem to indicate that hydralazine reacts with (at least) 2 types of "receptors" in oxygenated vascular smooth muscle, one vasodilator, which action appears at a relatively low concentration, and one inducing potentiation of vasoconstrictor stimuli, which action appears at a higher concentration and then counteracts the vasodilator component. It has as yet not been possible to elucidate the background of the interesting difference between the responses to hydralazine in bovine mesenteric artery under aerobic and anaerobic conditions.

Investigations in animals of the hypotensive response to increasing dosage of hydralazine indicate that even high doses of the drug produce only a moderate reduction of the blood pressure; further, the hypotensive response increases with increasing dosage up to 0.5—1.0 mg/kg (CRAVER et al. 1951) but higher doses do not produce a stronger response (see also GROSS et al. 1950, WALKER et al. 1951 and BEIN et al. 1953). These findings may indicate that the *in vitro* studies discussed above have a bearing also on the vascular

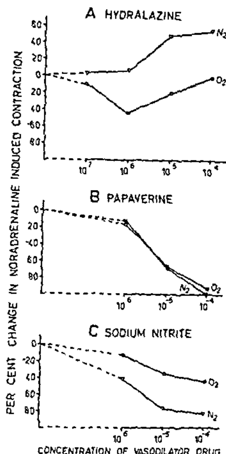


Figure 3 Effect of hydralazine (A), papaverine (B) and sodium nitrite (C) on noradrenaline induced isotonic contraction of *circulatory muscle of strips of bovine mesenteric artery* (prepared according to LUNDHOLM & MOHME LUNDHOLM 1960) The strips were loaded with 10 grams and suspended in Kreb's bicarbonate solution (containing 0 1 per cent glucose) at 37°C, bubbled with a mixture of 93 5 per cent O_2 and 6 5 per cent CO_2 (experiments indicated O_2) or 93 5 per cent N_2 and 6 5 per cent CO_2 (experiments indicated N_2) *Hydralazine was studied in 14 experiments, papaverine and sodium nitrite in 6* experiments In each experiment 8–12 strips from the same artery were studied simultaneously, half of them suspended in O_2, the other half in N_2 One hour after the strips had been suspended, noradrenaline (concentration varying in different experiments *from* 2.5×10^{-7} *to* 1×10^{-6}) was added *simultaneously to all strips The induced con*centration levelled off within 5 minutes and the shortening at this stage was recorded *The strips were washed and to half of them one of the vasodilator drugs was added in* the lowest concentration indicated on the abscissa Thirty minutes later the strips had relaxed and noradrenaline was again added in the same amount as before The produced shortening was recorded as before The strips were washed and the vasodilator drug was added to the same strips as before, now in 10 times higher concentration After another 30 minutes noradrenaline was again added In this way the experiment was continued until the highest concentration (10^{-4}) of the vasodilator drug had been studied The

indicated on the ordinate

effects of the drug *in vivo* although the relative importance of the different receptors in the vascular smooth muscle may be modified As suggested above in section 2 it also seems possible that the effects of a high intra arterial dose of hydralazine on the renal blood flow reported by MOYER (1959) can be explained in the light of these *in vitro* studies

4 *The mechanism for the hydralazine induced depression of "myogenic" vascular tone* The mechanism by which hydralazine reduces the "myogenic tone of vascular smooth muscle does not yet appear to have been conclusively demonstrated

As the hypotensive action of the drug is characterized by a slow onset it is possible that the vasodilator effect is not due to the hydralazine molecule *per se* but either to some metabolite of the drug or to some endogenous metabolite Should the vasodilator effect be due to such a more indirect action the active metabolite ought to be formed in the peripheral vasculature, where its action would be strictly local such a circumstance would be best reconciled with the peripheral effects demonstrated in communications I and II and the effects of hydralazine in *in vitro* vascular preparations

At present there seems to be only one metabolite of hydralazine identified namely acetylhydralazine (DOUGLASS & HOGAN 1959) This substance was found to be devoid of hypotensive action in the anesthetized cat when given in doses up to 2 mg/kg intravenously (own experiments)

Hydralazine has been found to be a potent *in vitro* inhibitor of histaminase (GROSS *et al* 1952 SCHULER & MEIER 1955 WERLE *et al* 1955) and a histamine liberator of low potency (PATON 1957) In view of these findings it might seem possible that the vasodilator action of hydralazine is due to an increased amount of free histamine especially as some side effects of the drug have been suggested to be due to such mechanisms (e g SCHROEDER 1953 PATON 1959 p 364) However certain observations seem to contradict the possibility that these mechanisms are of predominant importance for the vasodilator effect of the drug The hypotensive effect of hydralazine in animals was not reduced by antihistaminic drugs and hydralazine did not potentiate the hypotensive effect of intravenously injected histamine (BEIN *et al* 1953) Histaminase was potently inhibited *in vitro* also by hydrazine compounds which are chemically related to hydralazine but devoid of its prolonged hypotensive action (GROSS *et al* 1952 SCHULER & MEIER 1955) Hydralazine antagonized the vasoconstrictor action of histamine in isolated vessels (BEIN *et al* 1953 KIRPEKAR & LEWIS 1957 TRIPOD & MEIER 1958) The histamine liberator 48/80 constricted the coronary vessels of the isolated rabbit heart whereas hydralazine produced a dilatation (JAQUES *et al* 1957) When injected intradermally in man in doses up to 1 mg hydralazine did not produce

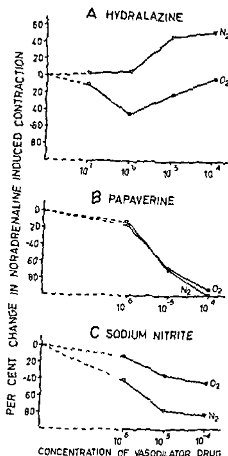


Figure 3. Effect of hydralazine (A), papaverine (B) and sodium nitrite (C) on noradrenaline induced isotonic contraction of circulatory muscle of strips of bovine mesenteric artery (prepared according to LUNDHOLM & MOHME LUNDHOLM 1960). *The strips were loaded* with 10 grams and suspended in Kreb's bicarbonate solution (containing 0.1 per cent glucose) at 37°C, bubbled with a mixture of 93.5 per cent O_2 and 6.5 per cent CO_2 (experiments indicated O_2) or 93.5 per cent N_2 and 6.5 per cent CO_2 (experiments indicated N_2). Hydralazine was studied in 14 experiments, papaverine and sodium nitrite in 6 experiments. In each experiment 8–12 strips from the same artery were studied simultaneously, half of them suspended in O_2, the other half in N_2. One hour after the strips had been suspended, noradrenaline (concentration varying in different experiments from 2.5×10^{-7} to 1×10^{-6}) was added simultaneously to all strips. The induced concentration levelled off within 5 minutes and the shortening at this stage was recorded. The strips were washed and to half of them one of the vasodilator drugs was added in the lowest concentration indicated on the abscissa. Thirty minutes later the strips had relaxed and noradrenaline was again added in the same amount as before. The produced shortening was recorded as before. The strips were washed and the vasodilator drug was added to the same strips as before, now in 10 times higher concentration. After another 30 minutes noradrenaline was again added. In this way the experiment was continued till the highest concentration (10^{-4}) of the vasodilator drug had been studied. The shortening produced in the drug treated strips was related to the simultaneous shortening in the untreated strips. The mean per cent change induced by the vasodilator drug is indicated on the ordinate.

SECTION 4

Comparative effects of intra arterially administered hydralazine and sodium nitrite on resistance and capacitance vessels in the forearm

a *General considerations* Previous sections have primarily dealt with the effects of hydralazine on peripheral vascular resistance However a vascular bed does possess a much more complicated function than merely controlling the resistance to blood flow In fact each circuit can be divided into several functionally differentiated "series coupled" sections like "Windkessel resistance sphincter shunt capacitance and exchange vessels (for definitions physiological details and references see MELLANDER 1960) For the understanding of the mechanisms of the general hemodynamic effects of a vasodilator drug it is necessary to have information on its effects especially on resistance and capacitance vessels The resistance vessels determine changes in the resistance to blood flow This function is predominantly exerted by smaller arteries and arterioles (precapillary resistance section) but to some extent also by venules and veins (postcapillary resistance section) In the capacitance vessels (predominantly venules and veins) changes of smooth muscle tone will alter the peripheral vascular blood capacity Such changes may influence the venous return of blood to the heart thereby adjusting cardiac output

It has been suggested that hydralazine exerts a dilator action on the arterioles but not on the veins (SCHMID & KELLNER 1953) Another hypotensive drug with a direct action on vascular smooth muscle sodium nitrite has been suggested to predominantly dilate the veins but only slightly the arterioles (WILKINS *et al* 1937) These suggestions were based on observations of the circulatory effects of systemically administered hydralazine or sodium nitrite and the mechanisms behind the proposed differentiated vascular response patterns have not been conclusively elucidated Because the cardiovascular response to hydralazine in certain respects is different from that of sodium nitrite (see section 5) it has been questioned whether the hemodynamic effects of hydralazine are due only to a "direct action on vascular smooth muscle (FREIS 1959 p 363) Therefore it seemed to be of interest to compare the effects of hydralazine and sodium nitrite on the resistance and capacitance vessels by a more direct approach

b *Experimental* An attempt was made to compare the effects of intra arterially infused hydralazine and sodium nitrite on the resistance and capa

a characteristic triple response but only a localized redness of the skin (own observation) according to PATON (1957) this method is a very useful for testing histamine release

Hydralazine has also been found to inhibit other enzymes *in vitro* such as dopa decarboxylase histidine decarboxylase and monoamine oxidase (WERLE *et al* 1955) and it has been found to enter into a great number of chemical and biochemical reactions (see SCHROEDER 1959) At present however it seems to be unknown if any of these reactions can be connected with the vasodilator action of the drug

The effects of various compounds chemically related to hydralazine have been studied (GROSS *et al* 1950 WALKER *et al* 1951 GROSS *et al* 1952 DRUEY & MARXER 1959 SCHULER & WYSS 1960) but the results do not appear to permit more definite conclusions concerning the structure activity relationships It seems possible that a hydralazine like vascular reaction is only exerted by compounds which beside a hydrazine moiety possess a chemical configuration allowing the formation of strong complexes with heavy metals (cf FALLAB & ERLENMEYER 1957)

It has been suggested that hydralazine is a general depressant of the oxidative energy metabolism and that the vasodilator effect of the drug could be due to such an action in the vascular smooth muscle cells (KIRPEKAR & LEWIS 1958 b 1959) Conclusive evidence in favour of this hypothesis has not yet however been forthcoming

effect on the blood flow in the human forearm Furthermore it was also found in this study that hydralazine produced a greater increase of the regional blood flow but a smaller increase of the total volume of the region than sodium nitrite These observations indicated that the peripheral vascular response patterns for the two drugs were fairly similar in the hind part of the cat and the human forearm

In the cat study hydralazine was found to produce a pronounced dilatation of the resistance vessels but only a slight dilatation of the capacitance vessels In comparison sodium nitrite evoked a distinctly weaker dilatation of the resistance vessels but a much more pronounced dilatation of the capacitance vessels It was further found that also the capillary fluid exchange was differently influenced by the two drugs Hydralazine elicited a net outward capillary filtration into the extravascular space Under the experimental conditions of the study a change in capillary fluid filtration transfer could be explained only by a change in mean hydrostatic capillary pressure This in turn could only be due to a change in the relation between precapillary resistance and postcapillary resistance Therefore hydralazine evidently had increased the mean hydrostatic capillary pressure indicating that the drug had produced a relatively more pronounced decrease of the precapillary resistance than of the postcapillary resistance This interpretation might be further supported by the finding that hydralazine predominantly dilated the resistance vessels (the most important ones situated on the precapillary side) while it only to a slight degree dilated the capacitance vessels (mainly postcapillary probably including also the postcapillary resistance vessels) — Sodium nitrite on the other hand did not significantly influence the net transcapillary fluid transfer This observation indicated that this drug so dilated the precapillary and postcapillary resistance vessels that the mean hydrostatic capillary pressure was essentially unchanged This would mean that sodium nitrite in comparison to hydralazine produced a relatively small decrease of the precapillary resistance but a relatively great decrease of the postcapillary resistance In agreement with this interpretation was the finding that sodium nitrite produced a smaller dilatation of the resistance vessels (mainly precapillary) but a greater dilatation of the capacitance vessels (mainly postcapillary) than hydralazine

c *Conclusions* In view of these findings it seemed possible to make a more conclusive interpretation of the results of the investigation in the human forearm The mean hydrostatic capillary pressure in the forearm was most likely relatively higher after infusion of hydralazine than after infusion of sodium nitrite Consequently hydralazine should have produced a relatively greater increase of extravascular fluid volume than sodium nitrite This would imply that the capacitance response of the two drugs presumably differed

citance vessels in the human forearm (IV) The effect on resistance vessels was estimated by recording the forearm blood flow with venous occlusion plethysmography The effect on capacitance vessels was deduced from the changes of the continuously recorded forearm volume The two phenomena were studied separately

Hydralazine and sodium nitrite were administered in low doses which produced no evident systemic effects The two drugs were found to produce an increase of both the blood flow and the total volume of the forearm and for each drug these effects were fairly well co ordinated in time in relation to each other When a quantitative comparison was made between the responses of the two drugs it was found that hydralazine produced a greater increase of the blood flow but a smaller increase of the total volume of the forearm than sodium nitrite These results indicated that the two drugs produced different vascular response patterns in the forearm In comparison to sodium nitrite, hydralazine appeared to evoke a more pronounced dilatation of the resistance vessels but a weaker dilatation of the capacitance vessels

However the results of this study alone could not be regarded as conclusive evidence of the interpretation made It has recently been shown (MELLANDER 1960) that vasoconstrictor and vasodilator stimuli may change the mean hydrostatic capillary pressure Such changes influence the filtration exchange across the capillary walls and alter the extravascular fluid volume Consequently, it might have been irrelevant to deduce the capacitance response from the induced changes of total forearm volume as these changes to some extent could be due to changes in extravascular fluid volume Therefore a complementary study was performed in the anesthetized cat (ÅBLAD & (MELLANDER 1963)

In this study a technique was utilized which permitted simultaneous and quantitative recordings of the resistance and capacitance responses as well as the filtration rate across the capillary walls in a skeletal muscle preparation in the hind part of the cat The effects on the resistance vessels were recorded by measuring the venous outflow of blood from the region where the arterial inflow pressure and venous outflow pressure were kept approximately constant The effects on the capacitance vessels were recorded by measuring the changes in regional blood content For this purpose the red blood cells of the cat were labelled with Cr^{51} The changes in radiation from the region as measured by an external scintillation detector were found to give a satisfactory estimate of the changes in regional blood content The effects on net transcapillary fluid movement were estimated by measuring the difference between the changes of the regional blood volume and the simultaneously occurring changes of the total regional volume which were recorded with a plethysmographic technique Hydralazine and sodium nitrite were infused intra arterially to the studied region in low doses and as a rule they did not significantly influence the systemic arterial blood pressure

The results showed that the two drugs increased the regional blood flow and for each drug this effect seemed to be well co ordinated in time with the

SECTION 5

Comparison between the general hemodynamic effects of hydralazine and sodium nitrite in man

a *General considerations* If the vascular reactions to hydralazine and sodium nitrite found in the forearm (section 4) represent the general vascular response pattern of the systematic circulation in man hydralazine should cause a greater decrease of the total peripheral vascular resistance but a smaller increase of the total peripheral vascular blood capacity than sodium nitrite If so the general hemodynamic effects of the two drugs should differ in certain respects because of different actions on the total peripheral resistance and the venous return of blood to the heart

However in the intact organism the drug induced changes of the arterial and venous pressures might modify the primary vascular effects of the drugs and their hemodynamic consequences For instance a reduction of the arterial pressure could — via the baroceptor reflex — be expected to evoke an increased discharge in the sympathetic vasoconstrictor nerves to both resistance and capacitance vessels (cf review by HEYMANS & NEIL 1958 FOLKOW 1962 b) This reflex mechanism would tend to oppose the peripheral [illegible]

[illegible] increased vasoconstrictor fibre discharge might modify the primary vasodilator effects considerably — A drug induced decrease of the arterial blood pressure might also — via the baroceptor reflex — be expected to influence the autonomic nerve control of the heart activity Such a reflex pattern involves a positive chronotropic effect (review by HEYMANS & NEIL 1958) and probably also a positive inotropic effect (NEIL 1962) This reflex mechanism would tend to increase the cardiac output if the venous return was sufficient

The integrated cardiovascular response to a vasodilator drug will depend upon many interconnected factors whose relative importance may be expected to vary widely in different individuals and in different situations To be able to establish if hydralazine and sodium nitrite produced different peripheral vascular response patterns in the intact organism one therefore had to compare their cardiovascular effects in the same subject under standardized experimental conditions The results of such experiments will be reported below (under b c and d) On the basis of these results the effects of the two drugs on the total peripheral resistance and blood capacity will be

more than was indicated by the difference in increase of total volume of the forearm Therefore the observation that hydralazine produced a more pronounced decrease of the vascular resistance but a less pronounced increase of the total volume in the forearm could only mean that hydralazine — in comparison to sodium nitrite — had a stronger relaxing action on the resistance vessels but a weaker relaxing action on the capacitance vessels

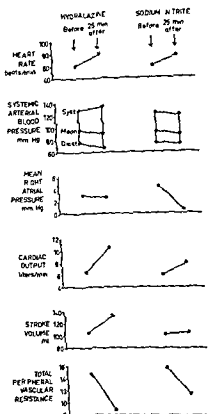


Figure 4 Mean effects in three subjects of intravenously infused hydralazine and sodium nitrite on heart rate, systolic, diastolic and mean intra arterial pressure, mean right atrial pressure, cardiac output, stroke volume and total peripheral vascular resistance The total peripheral vascular resistance estimated by dividing mean arterial blood pressure (mm Hg) by cardiac output (liters/min)

compared (under e) and the mechanism for their general hemodynamic effects will be discussed (under f) The interpretations made should be considered only as a basis for a working hypothesis partly because of the few experiments performed partly because the cardiovascular adjustments to a vasodilator stimulus are complex and insufficiently clarified

b *Comparative effects of intravenously administered hydralazine and sodium nitrite on total peripheral vascular resistance and cardiac output* If hydralazine in the intact organism evoked more dilatation of the peripheral resistance vessels but less dilatation of the peripheral capacitance vessels than sodium nitrite one would expect the total peripheral vascular resistance to be lower after hydralazine than after sodium nitrite at equal reduction of arterial blood pressure Cardiac output on the other hand should be relatively higher after hydralazine as this drug should reduce the "venous return" less than sodium nitrite To test this hypothesis the effects of intravenously infused hydralazine and sodium nitrite on the cardiac output heart rate arterial and right atrial blood pressure were compared (SANNERSTEDT & ÅBLAD unpublished)

Experiments were performed on 3 fasting healthy students in the recumbent position Cardiac output was determined by dye dilution technique using bromsulphalein The dye was injected into the right atrium and blood was collected from a brachial artery The techniques used have been described previously (WASSÉN 1956 BOJS 1961) The experiment was started about half an hour after the insertion of the catheters when the arterial and right atrial pressure and the heart rate were stable

In each subject two experiments were performed with an interval of at least one week In one experiment hydralazine was infused intravenously over a period of 5 minutes in a total dose of 0 2 mg/kg body weight In the other experiment sodium nitrite was infused intravenously for about 30 minutes at a rate of 0 15 mg/min/kg body weight Before drug administration cardiac output was determined twice in two subjects and once in one subject About 25 minutes after the start of the drug infusion cardiac output was again determined

Hydralazine evoked a gradual decrease of diastolic and mean arterial blood pressure and an increased heart rate These effects reached a relatively steady state about 15—20 minutes after the start of the drug infusion The infusion of sodium nitrite also produced a gradual decrease of the arterial blood pressure and an increased heart rate These effects disappeared fairly rapidly after the end of the infusion

Figure 4 shows the mean results obtained in the three subjects before and about 25 minutes after the start of drug infusion The two drugs produced about the same decrease of mean arterial blood pressure and approximately

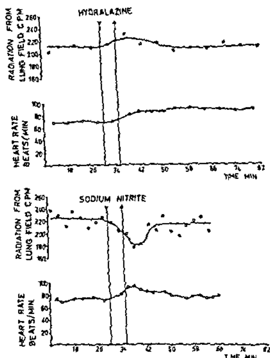


Figure 5 Effects of intravenously administered *hydralazine* (total dose 15 mg) and sodium nitrite (total dose 135 mg) on *plasma volume* in upper part of right lung and on heart rate After 2 hours rest in supine position the subject was given an i v injection of I^{131} albumin and recordings of the radiation from the lung field were started 10 minutes after the injection The scintillation detector was connected via an amplifier and a pulse height selector to a scaler (A B Nukleonikinstrument Sweden) The pulse height selector was set to reject all of the radiation outside the iodine photopeak In the experiment with hydralazine the radiation was recorded for five minute periods in the experiment with sodium nitrite for two minute periods — The "resting" level of radiation from the region was recorded for 20 minutes Thereafter hydralazine or sodium nitrite were infused i v for 5 minutes — Notice that sodium nitrite evoked a decreased radiation and an increased heart rate of short duration these changes were most pronounced just after the end of the infusion of the drugs (cf blood pressure changes evoked by similarly administered sodium nitrite in fig 6) Hydralazine produced no certain change of the radiation from the lung field but a gradual increase of heart rate

the same increase of heart rate Arterial pulse pressure increased after hydralazine but decreased after sodium nitrite Mean right atrial pressure decreased slightly after hydralazine but considerably more after sodium nitrite Cardiac output and stroke volume increased much more after hydralazine than after sodium nitrite Total peripheral vascular resistance decreased more after hydralazine The described differences between the effects of the two drugs were found in all three subjects studied However the changes produced by each of the drugs varied in certain respects in the individual experiments Thus hydralazine elicited an increase of the mean right atrial pressure in one subject but a decrease in the other two Sodium nitrite evoked a small increase of the stroke volume in two subjects but a decrease in the third

The different cardiovascular effects produced by hydralazine and sodium nitrite were consistent with the hypothesis that hydralazine dilated the peripheral resistance vessels more but the peripheral capacitance vessels less than sodium nitrite

c *Comparative effects of intravenously administered hydralazine and sodium nitrite on pulmonary blood volume* It has been found that an increase of peripheral vascular blood capacity tends to decrease blood pressure in central veins as a consequence part of the pulmonary blood volume tends to be redistributed to the systematic circulation (see reviews by SJOSTRAND 1953 1956) It was considered of interest to compare the effects of intravenously administered hydralazine and sodium nitrite on pulmonary blood volume If hydralazine produced a smaller increase of the total peripheral vascular capacity than sodium nitrite it should decrease the pulmonary blood volume less than an equihypotensive dose of sodium nitrite

This hypothesis was tested by the technique of WEISSLER *et al* (1959) The subject was given an intravenous injection of 50 μC I^{131} labelled serum albumin From 10 minutes after the injection changes of the plasma volume in the upper part of the right lung were studied by recording of the γ radiation from the region utilizing an external scintillation detector with a focusing collimator attachement Two experiments were performed on one healthy supine fasting subject (ÅBLAD HENNING JOHNSSON unpublished observations) In one experiment hydralazine (0 2 mg/kg infused intravenously for 5 minutes) was first given and about one hour later sodium nitrite (1 8 mg/kg infused i v for 5 minutes) was administered In the other experiment the drugs were given in reversed order but the doses were the same

Figure 5 shows the effects of hydralazine in the first experiment and those of sodium nitrite in the second experiment Both drugs produced about the same peak increase of the heart rate

the radiation from the lung field in

duced in both experiments a decrease of the recorded radiation This effect

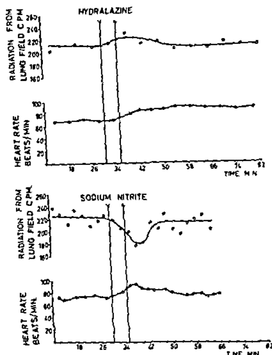


Figure 5 *Effects of intravenously administered hydralazine (total dose 15 mg) and sodium nitrite (total dose 135 mg) on plasma volume in upper part of right lung and on heart rate* After 2 hours rest in supine position the subject was given an i v injection of I^{131} albumin and recordings of the radiation from the lung field were started 10 minutes after the injection The scintillation detector was connected via an amplifier and a pulse height selector to a scaler (A B Nukleonikinstrument Sweden) The pulse height selector was set to reject all of the radiation outside the iodine photopeak In the experiment with hydralazine the radiation was recorded for five minute periods in the experiment with sodium nitrite for two minute periods — The "resting" level of radiation from the region was recorded for 20 minutes Thereafter hydralazine or sodium nitrite were infused i v for 5 minutes — Notice that sodium nitrite evoked a decreased radiation and an increased heart rate of short duration these changes were most pronounced just after the end of the infusion of the drugs (cf blood pressure changes evoked by similarly administered sodium nitrite in fig 6) Hydralazine produced no certain change of the radiation from the lung field but a gradual increase of heart rate

seemed to disappear more rapidly than the effects of the drug on heart rate and arterial blood pressure (cf fig 6)

These pilot studies indicate that pulmonary blood volume was essentially unchanged by hydralazine but decreased by sodium nitrite, thus supporting the hypothesis that hydralazine evoked a smaller increase of peripheral vascular blood capacity than sodium nitrite It has previously been found that thoracic blood volume was decreased by amylnitrite (SJOSTRAND 1941) and by nitroglycerin (SJOSTRAND 1951)

The pulmonary arterial pressure has been found to be increased by intravenously administered hydralazine in healthy subjects (WILKINSON *et al* 1952) This may be a consequence of the markedly increased cardiac output (AVIADO 1960) The pulmonary arterial pressure has been found to be decreased by sodium nitrite (FREIS *et al* 1949), nitroglycerin (BRACHFELD *et al* 1959) and erythrol tetranitrate (ROWE *et al* 1961) This change may also largely be a 'passive' effect and due to a decrease of pulmonary blood volume in the presence of a decreased or essentially unchanged cardiac output

The suggested explanation for the effects of hydralazine and nitrites on the pulmonary circulation is based on their actions on the cardiac output and on the systematic circulation It is possible that the drugs also influence the contractility of the pulmonary vessels but such an action does not yet appear to have been conclusively demonstrated (see review by AVIADO 1960)

d *Comparative effects of intravenously administered hydralazine and sodium nitrite on total blood volume* Hydralazine and sodium nitrite produced different effects on transcapillary fluid exchange in the skeletal muscle of the cat (cf section 4 b) It was therefore of interest to investigate if intravenous administration of the two drugs produced different effects on total plasma water volume (and total blood volume) As it seems possible to get a relatively good conception of acute changes of the plasma water volume by measuring the changes of hematocrit and of the concentrations of blood hemoglobin and serum protein (THOMPSON *et al* 1928, FINNERTY *et al* 1958 FAWCETT & WYNN 1960) serial determinations of these constituents were made before and after intravenous administration of hydralazine and sodium nitrite In addition arterial blood pressure was recorded

Experiments were performed on 5 healthy fasting students in recumbent position For sampling of blood and infusion of drugs a "Rochester plastic needle" (KNUTH *et al* 1958) was inserted in a subcutaneous cubital vein For recording of intra arterial pressure a teflon catheter (inner diameter 0 8 mm) was introduced into a brachial artery in 2 subjects In these 2 subjects the electrically integrated mean arterial blood pressure was recorded by a capacitance pressure transducer connected to a direct writing oscillograph (Mingograph Elema Co Sweden) In the other 3 subjects the auscultatory arterial blood pressure was measured according to RIVA ROCCI the diastolic pressure determined at

the point where the sounds suddenly began to fade. An approximate estimation of mean arterial blood pressure in these subjects was obtained by adding 1/3 of the pulse pressure to the diastolic pressure. Hematocrit measurements were carried out in capped Wintrobe tubes which were centrifuged for 55 minutes with a force of 1500 × g (CHAPLIN & MOLLISON 1952). The concentration of hemoglobin was determined spectrophotometrically after conversion to cyanmethemoglobin as described by HAINLINE (1958) (modification of the method of DRABKIN & AUSTIN 1935). The serum protein concentration was determined with a standard micro Kjeldahl technique.

Before each experiment the subject rested in supine position for at least 2 hours. On each subject two experiments were performed with an interval of at least one week. In one experiment hydralazine was administered intravenously for 5 minutes in a total dose of 0.2 mg/kg. In the other experiment sodium nitrite was infused intravenously for 5 minutes in a total dose of 1.8 mg/kg. Venous blood samples (each about 4 ml) were taken without venous occlusion with a 5—10 minute interval 3 times before and 6 times after the administration of the drugs. In these samples double determinations were made of hematocrit, hemoglobin and serum protein concentrations.

The mean results obtained in the five subjects are shown in figure 6. Hydralazine evoked a slowly developing reduction of the blood pressure that levelled off about 10 minutes after the end of the infusion and was essentially unchanged when the experiment was interrupted 35 minutes later. The mean decrease of the blood pressure at steady state level was about 7 mm Hg. Simultaneously with the development of the hypotensive response there occurred a slight increase of the hematocrit and of the concentrations of hemoglobin and serum protein. From 10 to 35 minutes after the end of the drug infusion the hematocrit was on an average 0.7±0.32[1]) per cent higher than before the infusion, the hemoglobin concentration was 1.7±0.32 per cent higher ($P<0.001$) and the serum protein concentration was 1.0±0.55 per cent higher.

Sodium nitrite (Fig. 6) evoked an almost immediate reduction of the arterial blood pressure. The hypotensive effect was maximal just after the end of the infusion when the average decrease of the mean blood pressure was about 6 mm Hg. Thereafter the blood pressure gradually returned to the pre-infusion level. There occurred a decrease of the hematocrit and of the hemoglobin and serum protein concentrations. The decrease was most pronounced immediately after the end of the infusion but some effect remained even 40 minutes later. Immediately and 5 minutes after the end of the infusion the mean hematocrit was 1.5±0.29 per cent lower than before the infusion ($P<0.001$), the hemoglobin concentration was 1.9±0.51 per cent lower ($P<0.001$) and the serum protein concentration was 2.4±0.46 per cent lower ($P<0.001$).

[1]) Mean and standard error of the mean calculated according to FISHER (1958).

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[1]) Mean and standard error of the mean calculated according to FISHER (1958)

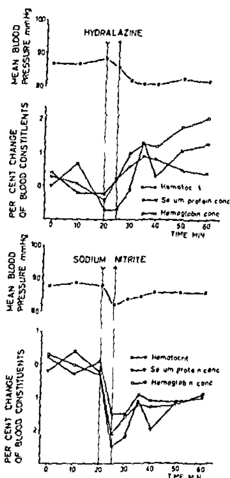


Figure 6 Mean effects of intravenously administered hydralazine (total dose 0 2 mg/kg) and sodium nitrite (total dose 1 8 mg/kg) on mean arterial blood pressure, hematocrit, hemoglobin and serum protein concentrations in five subjects Effects on the three blood constituents indicated as per cent change of mean pre infusion levels, which were within established normal limits in all subjects — Notice that hydralazine evoked a slowly developing reduction of mean arterial blood pressure, accompanied by slightly increased hematocrit, hemoglobin and serum protein concentrations Sodium nitrite produced a more rapid blood pressure reduction, accompanied by decreased hematocrit hemoglobin and serum protein concentrations

As all three blood constituents changed in the same direction after each of the drugs it was probable that the changes reflected changes of the total plasma water volume It was probable that plasma water volume (and total blood volume) tended to decrease slightly after hydralazine but to increase slightly after sodium nitrite By use of a dye dilution method SCHMID & KELLNER (1953) found that plasma volume decreased 380 ml on an average in 5 subjects given 16 mg hydralazine intravenously

As the drug induced blood volume changes developed fairly rapidly it seems probable that they reflected a change of the balance between the intravascular and extravascular fluid compartments The different effects of hydralazine and sodium nitrite on the blood volume were of such a type as could be expected if their effects on the mean hydrostatic capillary pressure in the organism were characterized by the same difference as in the cat skeletal muscle (see section 4 b)

Therefore the above reported results may indicate that hydralazine — in comparison to sodium nitrite — lowered the total peripheral precapillary resistance relatively much but that it lowered the total peripheral postcapillary resistance relatively little This interpretation may be supported by the fact (see below under e) that hydralazine — in relation to sodium nitrite — in the intact organism seemed to produce a more pronounced dilatation of the peripheral resistance vessels (mainly precapillary cf section 4 a) but a weaker dilatation of the peripheral capacitance vessels (mainly postcapillary probably including the postcapillary resistance vessels)

e Comparison of the effects of hydralazine and sodium nitrite on total peripheral vascular resistance and capacity The results reported above (b) indicate that hydralazine produced a more pronounced decrease of total peripheral vascular resistance than an equihypotensive dose of sodium nitrite As it was not considered [illegible] of total peripheral [illegible] this factor had to b[illegible] the drug induced changes of total blood volume and pulmonary blood volume Although the changes of cardiac blood volume were not studied it seems probable that hydralazine did not much increase total peripheral vascular blood capacity as it tended to decrease total blood volume and did not significantly change pulmonary blood volume An equihypotensive dose of sodium nitrite probably increased total peripheral vascular blood capacity distinctly as it evoked an increase of total blood volume and a decrease of pulmonary blood volume The view that hydralazine evoked a smaller increase of total peripheral vascular blood capacity than sodium nitrite was further supported by the finding that cardiac stroke volume was distinctly increased by hydralazine but relatively less increased or even decreased by sodium nitrite

Therefore the results of the above reported studies indicate that hydralazine evoked a greater decrease of total peripheral vascular resistance but a smaller increase of total peripheral vascular blood capacity than an equi hypotensive dose of sodium nitrite Accordingly the two drugs seemed to produce differentiated vascular response patterns in the systemic circulation of the same type as found by a more direct approach in the human forearm (section 4) The findings may indicate that the cardiovascular response to the two drugs was primarily determined by their direct peripheral vaso dilator action

As intravenously administered hydralazine has been found to produce a differentiated vasodilator response in parallel coupled resistance sections (cf section 3 f) one would expect that also its capacitance response might be characterized by regional differences In anesthetized animals intravenously administered hydralazine has been found to have a relatively pronounced action on the resistance vessels in the splanchnic region (BEIN *et al* 1953) and it was shown by MARKS *et al* (1955) that the drug increased the splanchnic blood volume in anesthetized dogs Part of this increased regional vascular capacity might be due to a distention of the veins because of an increased venous pressure The drug may also be expected to produce a pressure depend ent distention of the capacitance vessels in certain regions in man for instance in the liver where the blood flow and the venous outflow pressure have been found to be increased by intravenously administered hydralazine (FREIS *et al* 1953)

f *Mechanism for the general hemodynamic effects of hydralazine and nitrites in man* On the basis of the reported findings a tentative description of the mechanisms for the general hemodynamic effects of hydralazine and sodium nitrite in man will be made

Intravenously administered hydralazine produced a slowly developing peripheral vasodilatation through a direct action on vascular smooth muscle thereby reducing arterial blood pressure This would lead to a reflex increase of the sympathetic discharge to the vessels and the heart The in creased vasoconstrictor nerve tone would only to a slight extent counteract the strong direct effect of hydralazine on the resistance vessels whereas it seemed to outbalance the weak direct dilator effect of the drug on the perip heral capacitance vessels In this situation the diastolic cardiac filling pressure would be well maintained Therefore the sympathetic activation of the heart could lead to a marked increase of the stroke volume and in combination with the increased heart rate the cardiac output could be considerably in creased — As mentioned in the introduction hydralazine as a rule has been found to produce a pronounced decrease of peripheral vascular resistance and an increased stroke volume and heart rate (see further section 6 a)

As mentioned in section 1 c there is evidence that the cardiac stimulating effect of hydralazine is due to an increased sympathetic activity to the heart It might be questioned whether this effect is merely a reflex adjustment secondary to the peripheral vasodilator action of the drug since it may appear in the absence of blood pressure reduction (GRIMSON 1952) and may outlast the hypotensive effect for several hours (SCHAPER & JAGENEAU 1961) In this connection it may be of interest to quote some observations of the acute hemodynamic effects elicited by an experimentally produced arterio venous fistula (FRANK et al 1955) This measure should evoke a decrease of total peripheral vascular resistance without much influencing total peripheral vascular blood capacity thus resembling the peripheral vascular response to hydralazine When studying the acute hemodynamic effects produced by arterio venous fistulae of varying sizes in anaesthetized dogs FRANK et al (1955) found that a decrease of total peripheral resistance up to 30 per cent could often be completely outbalanced by an increased cardiac output so that no change of mean arterial blood pressure occurred These findings may be considered as an indirect support for the view that the cardiac stimulation evoked by hydralazine predominantly is a reflex adjustment secondary to the peripheral vasodilator effect of the drug

Sodium nitrite also evoked peripheral vasodilatation reduction of arterial blood pressure and a reflexly increased sympathetic activity to the vessels and the heart The increased vasoconstrictor nerve tone would tend to counteract the weak direct dilator effect of the drug on the resistance vessels and therefore peripheral vascular resistance was decreased to a comparatively small extent by this drug The strong direct dilator effect of sodium nitrite on the peripheral capacitance vessels would probably be counteracted only to a relatively slight degree by the reflexly increased vasoconstrictor nerve tone Therefore total peripheral vascular blood capacity was distinctly increased after administration of sodium nitrite The drug evoked a mobilization of blood from the pulmonary circuit to the systemic circulation and increased total blood volume Thereby the organism was capable of maintaining a certain pressure gradient between the central veins and the right heart in spite of the drug induced dilatation of the peripheral capacitance vessels — The reflex stimulation of the heart evoked an increased heart rate and also an increased ventricular contractility This positive inotropic effect would lead to an increased stroke volume if the "venous return of blood to the ventricles was sufficient Sometimes the venous return was high enough to allow a slight increase of stroke volume sometimes it was reduced to such an extent that stroke volume was decreased by the drug Consequently sodium nitrite evoked a relatively small increase of cardiac output largely due to an increased heart rate

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This interpretation seems to be supported by previous investigations of the cardiovascular effects of sodium nitrite (WEISS & ELLIS 1933, STARR *et al* 1937, FREIS *et al* 1949, ZIFFER *et al* 1957). Most frequently sodium nitrite was found to produce a hypotensive response with a decreased stroke volume and a decreased or unchanged cardiac output. WILKINS *et al* (1937) found that after oral administration of sodium nitrite there occurred in the hand a decrease in venous tone and at the same time an increase in arteriolar tone. The latter effect was suggested to be due to a reflexly increased vasoconstrictor fibre discharge which outbalanced the weak direct arteriolar dilatation evoked by the drug.

As the vasodilator effect of alkylnitrates is generally considered to be similar to that of sodium nitrite one would expect that their cardiovascular response pattern should approximately correspond to that described above for sodium nitrite. Recent studies of the effects of sublingually administered nitroglycerin (BRACHFELD *et al* 1959) and erythrol tetranitrate (ROWE *et al* 1961) on stroke volume, heart rate and peripheral vascular resistance appear to support this presumption. The cardiovascular effects of nitroglycerin in man have been studied in several other investigations but with techniques that are probably less accurate (e g STARR *et al* 1937, WEGRIA *et al* 1951, BRANDT *et al* 1952, HOHNEN & MEESE 1960). The results of these studies are widely divergent. These variations may in part be due to the fact that sublingually administered nitroglycerin produces distinct and abrupt subjective symptoms and consequently the cardiovascular response to the drug may be expected to be modified to a marked and varying extent by emotional factors engaging cardiovascular sympathetic nerves (cf BRANDT *et al* 1952). To judge from some recent studies in anaesthetized animals (HONIG *et al* 1960) and man (PORJÉ & RUDEVALD 1962) it is also probable that the cardiovascular response to nitroglycerin may be highly different during the initial stage when the action develops and during a later stage when the action is at steady state or declining. Such a circumstance may also in part explain the different results obtained with this drug.

SECTION 6

Some comments on the hemodynamic effects of hydralazine in hypertensive disease

a *Acute hemodynamic effects of hydralazine* The aspects on the acute hemodynamic effects of hydralazine presented in previous sections have to a great extent been based on experiments in healthy normotensive subjects However many of the investigations discussed have been performed on hypertensive patients The hypotensive action of hydralazine in these patients is probably due to the same mechanism as in normotensive subjects The peripheral vascular response to intravenously administered hydralazine seems to be determined by a "direct" vasodilator action and an opposing increased vasoconstrictor fibre discharge both in normotensive and hypertensive subjects (see STUNKARD *et al* 1954 and experiments reported in section 1 b) As a rule intravenously administered hydralazine increases renal and hepatic blood flow in both normotensive and hypertensive subjects (e g WILKINSON *et al* 1952 FREIS *et al* 1953) but does not change the blood flow considerably in the calf in either group (FREIS *et al* 1953) This indicates that the drug produces in principle the same differentiated response pattern on the resistance in "parallel-coupled" vascular regions in both hypertensive and normotensive subjects The hypotensive effect of hydralazine is as a rule accompanied by a considerably increased stroke volume and cardiac output also in hypertensive subjects (FREIS *et al* 1953 STEIN & HECHT 1955 ROWE *et al* 1955 a JUDSON *et al* 1956 a) Therefore it seems probable that hydralazine in principle evokes the same response pattern as regards the peripheral resistance and capacitance functions both in hypertensive and normotensive subjects

However it is evident from the above mentioned studies that there is a great individual variation in the cardiovascular response to hydralazine in hypertensive patients This variation is probably to a great extent due to the fact that the cardiovascular system is more or less pathologically changed in these patients Sometimes intravenously administered hydralazine elicits a pronounced reduction of the arterial blood pressure to "shock" levels with decreased unchanged or increased cardiac output this response seems to occur in patients with low "cardiac reserve" (ROWE *et al* 1955 a)

Sometimes hydralazine has been found to decrease renal plasma flow in hypertensive patients (e g WILKINSON *et al* 1952 STEIN & HECHT 1955) This seems to occur partly in patients where the drug evokes a profound fall

of the arterial blood pressure partly in patients where the basal renal plasma flow is low. It has been suggested that the latter group is incapable of responding to the drug by a distinct renal vasodilatation because of irreversible vascular pathology (STEIN & HECHT 1955). A factor which might be of contributory importance — in view of the circumstances discussed above in section 3 g — is that the concentration of hydralazine in the renal vasculature might be relatively less in these patients than in patients with normal renal plasma flow.

Hydralazine is known to produce anginal pain in patients with coronary sclerosis (e.g. MOYER 1953). Intravenously administered hydralazine regularly evoked myocardial ischemic symptoms in patients with this syndrome (JUDSON *et al.* 1956 b). Hydralazine has been found to increase coronary blood flow and decrease coronary arterio-venous oxygen difference in hypertensive patients (ROWE *et al.* 1955 b). On the other hand heart work is increased by the drug if the reduction of arterial blood pressure is moderate (WILKINSON *et al.* 1952, ROWE *et al.* 1955 b). It is possible that hydralazine does not much reduce the resistance in a sclerosed coronary vascular bed and therefore the increased heart work will evoke myocardial ischemia in patients with the coronary sclerotic syndrome.

In contrast to hydralazine the nitrites-alkylnitrates are known to relieve or prevent angina pectoris. It has been questioned whether nitroglycerin increases the coronary blood flow in patients with angina pectoris (GORLIN *et al.* 1959). Erythrol tetranitrate has been found to decrease the work of the heart and this action was ascribed to a pronounced dilatation of the peripheral capacitance vessels (ROWE *et al.* 1961). The authors suggested that this extracardial point of action was of contributory importance for the beneficial effect of the drug in angina pectoris. — It seems possible that the marked difference between the effects of hydralazine and the nitrites-alkylnitrates on the coronary sclerotic syndrome is at least in part a consequence of their different peripheral vascular response patterns.

b. *Hemodynamic effects of hydralazine in long term therapy.* Double blind control studies have shown that hydralazine often is an effective antihypertensive agent in long term therapy (MERRIL & KENYON 1953, LEE *et al.* 1956, ARMSTRONG *et al.* 1960). The chronic hypotensive effect of hydralazine seems to a comparatively high degree to be due to a reduction of diastolic blood pressure (ARMSTRONG *et al.* 1960, BJORK *et al.* 1960, HOOD 1960, p. 301). It has been found that cardiac stroke volume, heart rate and renal plasma flow often are maintained at an increased level during chronic oral administration of the drug in hypotensive doses (JUDSON *et al.* 1957). These observations indicate that the circulatory effects of the drug in long term therapy are in principle similar to its acute effects.

c Concluding remarks Hydralazine is nowadays seldom used alone in antihypertensive therapy because of slight efficiency and distressing side effects of which one of the more important anginal pain appears to be related to its stimulating action on the heart In combination treatment, however, the drug has often been found to be an effective and well tolerated adjunct, when given in moderate doses (e g SCHROEDER 1959, BJÖRK *et al* 1960 FREIS 1961) The above presented views on the mechanism for the cardiovascular effects of hydralazine would seem to be consistent with this clinical experience When given alone, hydralazine should produce a decrease of peripheral vascular resistance because of its pronounced 'direct' action on peripheral resistance vessels However, this effect may be largely outbalanced by an increased cardiac output, due to increased sympathetic discharge to heart and peripheral capacitance vessels This latter effect should be weakened by agents which primarily antagonize cardiovascular sympathetic tone (e g ganglionic blocking agents and guanethidine) Therefore, hydralazine should be an effective hypotensive drug in combination with such agents

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Mr ANDRAS BEVIZ for drawing the illustrations

All student colleagues who have willingly served as test subjects

My wife GISELA for constant encouragement and support

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2 The vasodilator effect of intravenously administered hydralazine was predominantly due to a site of action in the peripheral vascular bed Sympathetic vasoconstrictor fibre discharge seemed to be increased probably representing a reflex adjustment to the vasodilator effect

3 When administered intra arterially in low dosage to the forearm and the hand hydralazine evoked a decrease of the regional vascular resistance characterized by slow onset and long duration This response was found to be due to depression of intrinsic myogenic vascular tone

4 A comparison was made of the effects of intra arterially administered hydralazine and sodium nitrite on the resistance and capacitance vessels of the forearm Of the two drugs hydralazine produced a more pronounced relaxation of the resistance vessels but a weaker relaxation of the capacitance vessels

5 A comparison was made of the effects of intravenously administered hydralazine and sodium nitrite on total peripheral vascular resistance and total peripheral vascular blood capacity in the systematic circulation The results indicate that hydralazine dilated the resistance vessels more but the capacitance vessels less than sodium nitrite Furthermore the general hemo dynamic effects of intravenously administered hydralazine and sodium nitrite were characterized by differences that could be ascribed to differentiated vascular response patterns in the general systematic circulation of the same type as found by a more direct approach in the forearm

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CALCIUM BINDING SUBSTANCES AND INTESTINAL ABSORPTION

A survey of literature and own investigations

BY

ERLING SØGNEN

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ACTA PHARMACOLOGICA ET TOXICOLOGICA

From the Veterinary College of Norway, Oslo
Department of Pharmacology and Toxicology
(Head Professor Dr Ottar Dybing)

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Furthermore, my thanks are due to the photographer, Mr. H. Giltvedt.

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CALCIUM AND INTESTINAL ABSORPTION OF GLUCOSE

The effects of calcium on absorption and cell permeability have been studied by several workers during the last thirty years The first investigation into the effect of the calcium ion on intestinal absorption was apparently carried out by MAGEE & SEN (1931) on isolated loops of rabbit intestine in vitro The segment was rinsed out, filled with Tyrode solution containing glucose and placed in beakers containing glucose-free Tyrode solution, which was continually oxygenated Even if it is likely that these preparations must have been inadequately oxygenated on the mucosal side *(Quastel* 1961), the experiments gave valuable informations, showing that glucose transport from lumen to the outer fluid increased when calcium was added to the latter Lively contractions were, however, observed in the preparations placed in Tyrode solution containing the calcium To exclude the possibility that the increase in intraluminal pressure caused by the contractions might have increased the glucose transport in presence of calcium, the authors added adrenaline to the outer fluid WIX *et al* (1952), however, have shown ...
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MAGEE & SEN (1932) also examined the effect of hypocalcaemia (induced by parathyroidectomy) on glucose absorption in rats in vivo, using the method of CORI (1925) It was found that glucose absorption was reduced in animals with hypocalcaemia The rate of glucose absorption was restored to normal values when the glucose was given in a 0 32 per cent solution of calcium chloride, but the same amount of calcium chloride inhibited glucose absorption in normal animals

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glucose absorption in rats Similar effects could not be demonstrated in hypocalcaemic rabbits with isolated loops in situ

GELHORN & SKUPA (1933) experimented with simultaneous perfusion of intestinal lumen and intestinal blood vessels in situ in toads The blood vessels were perfused with Ringer's solution, and the intestinal lumen with a solution containing glucose Under these experimental conditions glucose absorption was reduced in presence of calcium in the Ringer's solution perfusing the blood vessels

McDOUGALL (1935) in experiments with isolated loops found no reduction of glucose absorption in hypocalcaemic urethaneanaesthetized rats The glucose absorption was unaltered in absence or presence and at different concentrations of calcium chloride, and so was the absorption of water, chloride, xylose, and sorbose The author suggested the possibility that any apparent discrepancy between the result of MAGEE & SEN (1932) in hypocalcaemic rats and his own was due to an effect of stomach emptying in the experiments of the former authors

GARDNER & BURGET (1938) had noticed the apparently contradictory results of the experiments on the effect of calcium on glucose absorption In experiments on dogs with closed isolated loops by the method of BURGET (1934) they found that glucose was absorbed more slowly from the intestine in presence of calcium chloride at concentrations from 0 03 to 0 15 per cent than in absence of calcium chloride

In comparing the above-mentioned results, differences in experimental conditions must obviously be taken into consideration, thus variations in calcium concentration and calcium status of the animal may have been of importance

1) *Considering the experiments in which glucose absorption is studied* under conditions excluding the possible influence of stomach emptying and in which calcium concentration is varied in the intestinal lumen in vivo, it is seen that calcium reduces the rate of glucose absorption or leaves it unaltered

2) In experiments in which stomach emptying might be affected, glucose absorption has been unaltered or increased in presence of calcium

3) In experiments in which calcium concentration is increased in the intestinal blood vessels, glucose absorption is decreased

4) In isolated segments of rabbit intestine in vitro, glucose absorption is increased when calcium is added to the outer fluid

That calcium increases glucose transport in vitro was verified by DARLINGTON & QUASTEL (1953) They were interested in the problem, because their in vitro preparations showed lively peristalsis in presence of calcium ions In order to exclude this peristaltic movement, the authors preferred to work which calcium free solutions, and they found

that under their experimental conditions the removal of calcium from the in vitro system caused a fall in glucose transfer of about 30 % The preparation in question is an in vitro preparation with well-oxygenated outer and inner fluids

CSAKY & THALE (1960) also maintain that calcium and the ions of sodium or magnesium are of essential importance for glucose transport in vitro

*

The most striking effects of calcium binding substances (Ca b.s) on the intestine is that on intestinal water transport (SØGNEN 1965 c, d & e)

It is therefore natural to pose the following questions

1) Is there any relationship between the observed alteration in water transport caused by Ca b s and the reduced glucose transport in absence of calcium in vitro referred to above?

2) Of what nature is the connection between the altered water transport and the apparently retarded absorption of drugs from the intestinal tract in vivo? (SØGNEN 1961 & 1965 a)

The facts given below may be of some importance in these connections

The effect of calcium on intestinal transport of water and sodium

Simultaneously with our investigations DUMONT *et al* (1960) found that calcium exercises a drastic effect on active sodium transport from the intestine and on concomitant water movement Increasing the calcium concentration from 0 to 1 mM caused a sharp increase in sodium and water removal from the intestinal lumen At concentrations higher than 1 mM the effect of calcium ions was reversed, and sodium and water absorption decreased slowly as calcium concentration was increased

HEIDENHEIN (1894) was the first to demonstrate that sodium fluoride depresses the absorption of sodium chloride from the intestinal lumen He regarded this as due to competition between fluoride and chloride ion for the routes of transport

It would not be justifiable to discuss in detail any possible connection between these two observations, but it may be useful to associate them when considering some of the problems arising from our investigation

The effect of glucose on water transport

The above notes on the relationship between calcium and transport of glucose and of sodium and water appear to be of special interest in connection with the statements that water transport seems to depend on glucose metabolism and transport in vitro (FISHER 1954 & 1955, SMYTH & TAYLOR 1954 & 1957, LIFSON & PARSONS 1957, PARSONS & WINGATE 1961)

This interrelationship between water and glucose transfer was also convincingly demonstrated by BARRY, MATTHEWS & SMYTH (1961) Their experiments with everted loops of rat small intestine not only confirm the results of FISHER, SMYTH & TAYLOR, but go further than this by demonstrating that the water transfer can be divided into two fractions, one glucose dependent and one glucose independent The former appears to be more important in the upper part of the intestine, the latter is certainly the more important in the lower part of ileum

In a review on glucose transport SMYTH (1962) maintains «The relation of glucose absorption to water and electrolyte movement is of interest The absorption of fluid both in vivo and in vitro is known to be related to glucose uptake In conditions in vitro this is probably because metabolism of part of the glucose absorbed is essential for transfer If the fluid movement depends upon Na transfer, then clearly Na transfer is related to glucose movement This arrangement is, however, a reciprocal one, and glucose movement is in turn dependent upon the presence of Na This was first shown by RIKLIS & QUASTEL, and CRANE has suggested a tentative hypothesis to account for this As he himself says, the problem is complex, and awaits further experimental evidence Another aspect of intestinal absorption of glucose is its relation to the nutrition of the intestine The intestinal epithelium differs from all of the other cells in the body in that it has the first opportunity to use the glucose formed in the intestinal tract, and, furthermore, this is presented to it directly and not only by the blood stream » WILSON (1962) discussed the role of glucose in water transport, and concluded «that the only effect of glucose is an indirect one on the nutrition of the cell and, furthermore, only occurs with in vitro preparations of rat intestine »

The relation between transport of water and solutes

As stated by FISHER (1955), the transport of solutes of low molecular weight, e g urea and sorbose, depends on water transport

It has also been suggested by SMYTH & TAYLOR (1957) that one part of glucose transport might be due to passive transfer in an «active

waterstream», and a similar possibility has been suggested by BARRY & SMYTH (1960) for a part of the fatty acid transfer

On the basis of this literature survey it may be stated, first, that calcium seems to affect intestinal glucose and water transfer

Water transfer seems to depend on glucose transfer, and possibly calcium is somehow involved in this relationship, perhaps because it affects the transport of sodium ions

Solutes of small molecular weight depend on water for transfer across membranes, and their transport may, accordingly, be affected by the concentration of calcium

As a consequence removal of calcium from the intestinal lumen by means of Ca b s might be a primary cause for the apparently retarded absorption of drugs from the small intestine (SØGNEN 1963 & 1965 a).

This hypothesis will be discussed in connection with own experiments referred to later in this survey

CALCIUM BINDING SUBSTANCES

Sodium oxalate

Oxalic acid is a normally occuring metabolite, and is excreted in the urine of man in amounts of about 25 30 mg (FÜHNER 1943) It occurs in varying amounts in many plants A table showing the occurrence in a series of higher plants is given by KING & SPERRY (1961) The amount of oxalic acid may be about 1 % of the dry matter

Oxalate production by various species of common fungi was known as early as 1870 (FOSTER 1946) Such species, especially *Aspergillus flavus* and *niger*, occur in mouldy feedstuffs and may produce such quantities of oxalate that calcium is rendered unavailable Analyses of infected wheat, oats and Bermuda grass hay revealed oxalate well in excess of that theoretically required to precipitate all calcium present under optimal conditions (WILSON & WILSON 1961)

Sodium oxalate is absorbed from the intestinal tract Intestinal absorption of sodium oxalate must, however, depend on the amount of calcium present, since calcium oxalate is practically insoluble in the intestinal tract, and passes through it unchanged in cattle (TALAPATRA *et al* 1948) The same seems to occur in the rat. HAAVALDSEN *et al* (1956) added sodium oxalate to the rations in order to obtain a low calcium intake during experiments with vitamin D In experiments with rats

The effect of glucose on water transport

The above notes on the relationship between calcium and transport of glucose and of sodium and water appear to be of special interest in connection with the statements that water transport seems to depend on glucose metabolism and transport in vitro (FISHER 1954 & 1955, SMYTH & TAYLOR 1954 & 1957, LIFSON & PARSONS 1957, PARSONS & WINGATE 1961)

This interrelationship between water and glucose transfer was also convincingly demonstrated by BARRY, MATTHEWS & SMYTH (1961) Their experiments with everted loops of rat small intestine not only confirm the results of FISHER, SMYTH & TAYLOR, but go further than this by demonstrating that the water transfer can be divided into two fractions, one glucose dependent and one glucose independent The former appears to be more important in the upper part of the intestine, the latter is certainly the more important in the lower part of ileum

In a review on glucose transport SMYTH (1962) maintains «The relation of glucose absorption to water and electrolyte movement is of interest The absorption of fluid both in vivo and in vitro is known to be related to glucose uptake In conditions in vitro this is probably because metabolism of part of the glucose absorbed is essential for transfer If the fluid movement depends upon Na transfer, then clearly Na transfer is related to glucose movement This arrangement is, however, a reciprocal one, and glucose movement is in turn dependent upon the presence of Na This was first shown by RIKLIS & QUASTEL, and CRANE has suggested a tentative hypothesis to account for this As he himself says, the problem is complex, and awaits further experimental evidence Another aspect of intestinal absorption of glucose is its relation to the nutrition of the intestine The intestinal epithelium differs from all of the other cells in the body in that it has the first opportunity to use the glucose formed in the intestinal tract, and, furthermore, this is presented to it directly and not only by the blood stream » WILSON (1962) discussed the role of glucose in water transport, and concluded «that the only effect of glucose is an indirect one on the nutrition of the cell and, furthermore, only occurs with in vitro preparations of rat intestine »

The relation between transport of water and solutes

As stated by FISHER (1955), the transport of solutes of low molecular weight, e g urea and sorbose, depends on water transport

It has also been suggested by SMYTH & TAYLOR (1957) that one part of glucose transport might be due to passive transfer in an «active

Sodium phytate

Phytic acid is the hexaphosphoric acid of inositol $C_6H_6(O\ PO(OH)_2)_6$ (POSTERNAK 1921) and is thus a dodecabasic acid.

In plants it is found as an acid capable of forming a large number of salts Such salts of phytic acid containing various alkali and alkaline earth metals together with traces of iron, manganese and other nutritionally important mineral elements, are found at *especially high concentrations* in cereal grains (ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY VOL 7) According to PEDERSEN *(1940) 60* 85 per cent of the phosphorus in cereal occurs as phytic acid.

In an extensive investigation into the solubility of calcium phytate, HOFF-JØRGENSEN *(1944) found that, under* conditions *similar to those in* the intestine, calcium absorption is likely to be *impaired in* presence of large quantities of phytic acid Thus, in a solution of phytic *acid and* calcium ions amorphous pentacalcium phytate precipitates in the *pH* range *5-7, moreover it is* of importance that a considerable part of the dissolved calcium will be bound as a *complex in the presence* of phytic acid

Phytic acid is not absorbed from the alimentary canal (STARKENSTEIN 1910) *It combines with* calcium *in the intestine* to lower its availability to those animals that lack a *phytase in their* digestive tract (NICOLAYSEN *et al 1953) It has been shown to have* a decalcifying action in rats, puppies and humans (MELLANBY 1925, NICOLAYSEN & NJÅ 1951)

Sodium phytate has been *employed in cases of* hypercalcaemia due to vitamin D poisoning, *to sarcoid, and to certain* renal stone syndromes (HENNEMAN 1956)

ROBERTS & YUDKIN (1960) *made* a study of the effects of dietary phytate upon intestinal phytase and bone calcification in the rat Signs of phytate intoxication were *observed at levels* ranging from 1 to 10 per cent The rats developed *severe diarrh*oea within 24 hours and rapidly lost weight Within a week the *animals died* with symptoms apparently due to hypomagnesaemia This was verified by the fact that intoxication caused by sodium phytate could be prevented with magnesium, but not with calcium As phytate is inositol *hexaphosphate*, the authors excluded the possibility of inositol *deficiency*, due to competitive inhibition, by adding 50 mg inositol daily to the diet It is suggested that a dietary constituent such as phytate, *which* can render magnesium unavailable, may be a cause of hypomagnesaemia in cattle Phytate and diets *rich in* phytate considerable reduce *iron* absorption (SHARPE *et al* 1950) It must be pointed out, however, that the last named *authors have* found

KOHMANN (1939) found that spinach, although relatively high in calcium (0 12 %), contained so much oxalic acid (0 89 %) that there was still enough free oxalic acid (0 62 %) to render calcium from other foods unavailable SCHMIDT NIELSEN & SCHMIDT NIELSEN (1944) also considered calcium bound oxalate as not absorbed

It seems reasonable to suggest that only high single oral doses of sodium oxalate would be likely to reduce the serum calcium to hypocalcaemic levels Thus SCHMIDT NIELSEN & SCHMIDT NIELSEN (1944) saw no reduction in serum calcium after 10 days of feeding with spinach between meals to rats When, however, calcium balance had been negative for 20 days, serum calcium was significantly reduced by this means The spinach contained oxalic acid equivalent to half the daily intake of calcium Obviously the sodium oxalate was absorbed as such, causing aggregates of calcium oxalate in the renal tubules

Alterations in the dynamics of calcium metabolism have, however, been demonstrated in rabbits given radiocalcium intravenously after feeding spinach containing oxalic acid THOMAS *et al* (1954) found by this means that the intestinal calcium reserve of oxalic acid competed with the bone in the exchange process for the available radiocalcium after its intravenous injection It is therefore obvious, as with tetracemin (POPOVICI *et al* 1950) and sodium fluoride (FRIEDENTHAL 1901), that the amount of calcium binding substance brought into circulation per unit of time determines whether or not hypocalcaemia is induced This is true as long as calcium can still be mobilized from the bone reservoir in sufficient amounts to replace the serum calcium eliminated by the calcium binding substance

Apart from its use as an anticoagulant in vitro, sodium oxalate has no established medical use In small amounts in vivo, however, it does shorten rather than prolong coagulation time (STEINBERG & BROWN 1939), and has to a limited extent been applied in the surgical control of bleeding (BLAIN & CAMPBELL 1942)

Sodium oxalate has, however, been a useful tool in experimental pharmacology for the study of in vitro processes in calcium free media (POHL 1923, MAGEE & SEN 1932) and in creating experimental hypocalcaemia on intravenous injection (SEEKLES *et al* 1931, STEWART & BOWEN 1951, and PAYNE *et al* 1963)

The use of oxalate for such purposes has the disadvantage that calcium is precipitated in the organism or in the in vitro system studied In vivo this leads to the risk of kidney damage through calcium oxalate concretions As pointed out by PAYNE (1964), calcium takes about five minutes to be completely precipitated as calcium oxalate, which is then taken up by the reticulo endothelial system and not excreted via the kidneys

and accumulated in the hard tissues, whereas calcium chloride, which was studied simultanously, seemed to accumulate in soft tissues, such as some striated muscles

A comprehensive review of acute and chronic fluorine poisoning in man and animals was prepared by ROHOLM (1938)

The original conception of the toxic effects of fluoride is associated with its calcium depleting action through formation of insoluble calcium fluoride This view is supported by the fact that tolerance towards sodium fluoride increases with decreased rate of administration, indicating that calcium mobilization is an important detoxifying factor in fluoride poisoning (FRIEDENTHAL 1901) Further, as demonstrated by SCHLICK (1911), the administration of sodium fluoride simultaneously with an equivalent amount of calcium chloride did not cause intoxication in frogs or rabbits Finally, there are many common features in intoxication with fluoride and oxalate poisoning, for example the fall in the calcium concentration of the blood (JODLBAUER 1931)

On the other hand, the view has been maintained that all the effects of sodium fluoride cannot be caused by the calcium binding *properties of fluorine ions* Numerous experiments carried out on various organisms show that fluoride has biological effects apparently even in presence of a surplus of calcium ions in the system studied, and also has biological effects that are not produced by oxalate or citrate

Fluoride is known to inhibit enzymes requiring Ca, Mg, Mn, Zn, and Cu, such as enolase, certain esterases, and bone alkaline phosphatase (CANTAROW & SCHEPARTZ 1962)

For enolase inhibition, the mechanism is supposed to be that 2 phosphoglyceric acid undergoes reversible dehydration to phosphoenolpyruvic acid under the action of enolase *Mg^{++} is required* for the action of this enzyme Inhibition by fluorine is explained by the formation of magnesium fluorophosphate This *example is* cited in order to stress the possibility that [illegible]

[illegible] 1963 a,b,c,d,e,f & g

Tetracemin

Ethylenediamintetracetic acid (EDTA) forms water soluble complexes with many divalent metallic ions (SCHWARTZENBACH & ACKERMAN 1948) The chelation is preferential, and depends on the pH of the solution

that, though added soluble phytates can interfere with iron absorption, there was no correlation between the concentration of phytates in ordinary meals and the reduction in iron absorption The reduction in iron absorption caused by soluble phytates is thought to be due to precipitation of insoluble iron phytate (WIDDOWSON & McCANCE 1942, McCANCE *et al* 1943)

Sodium fluoride

In contrast to the salts of phytic and oxalic acid, fluorides in plants usually occur only in trace amounts The ability of plants to take fluorine up from the soil varies, but it is generally accepted that its amounts in most plants are not readily affected by the amount in the soil The high amounts of fluorine in most herbage on soil rich in it is assumed to be due to contamination with soil particles and not usually to its uptake by the plants (ALLCROFT 1954) Such contamination is also seen in industrial areas where pastures are exposed to fluorine containing gases (GARNER 1957)

Of particular interest in veterinary medicine is the rather high content (3 5 4 %) of fluorine in sedimentary rock phosphate, which is used as a source of phosphorus in animal nutrition The fluorine appears mainly as the complex salt apatite ($3Ca_3(PO_4)_2CaF_2$) (MORRISON 1948)

Sodium fluoride is used as an anthelmintic against ascaris infestation in swine It is given in single doses from 100 to 150 mg/kg, and added to the dry ground feed at a concentration of 1 % (HABERMANN *et al* 1945) In the recommended doses sodium fluoride can cause vomiting and the passage of soft faeces In larger doses diarrhoea was seen HANSEN (1957) points out the reduced utilization of feed and the reduced gains in weight associated with the use of sodium fluoride as an anthelmintic

COX (1952) published the first extensive review on the fluoridation of water supplies in caries prophylaxis The mechanism by which fluorine reduces the incidence of dental caries is unknown It is, however, believed that it may undergo surface adsorption by hydroxyapatite crystals of enamel, forming a protective layer of acid resistant fluoroapatite (CANTAROW & SCHEPARTZ 1962)

The fluorine ion is absorbed from the intestinal tract but calcium fluoride is poorly absorbed (DAVIS 1964) In experiments with rats it has been shown that covalently held fluorine is absorbed at a greater rate than the fluorine ion (ZIPKIN & LIKINS 1957)

Distribution studies carried out by APPELGREN *et al* (1961) revealed that F^{18} injected intravenously was taken up from the blood fairly rapidly

DENBLAD 1956) Thus calcium tetracemin is a valuable therapeutic agent in lead poisoning (BESSMAN *et al* 1952, RUBIN *et al* 1953, SIDBURY *et al* 1953)

The substance has also been shown to be effective in chelating radioactive elements, such as plutonium and radioyttrium, thereby expediting their excretion from the body

A series of other chelating substances (which may well have effects on the intestinal tract similar to those of tetracemin) are under investigation for the purpose of finding a substance that will bind strontium preferentially to calcium, in order to retard the absorption or accelerate the excretion of radiostrontium KROLL & GORDON (1960) have reported a selectively favourable chelate binding for strontium of certain alkylene homologues of tetracemin

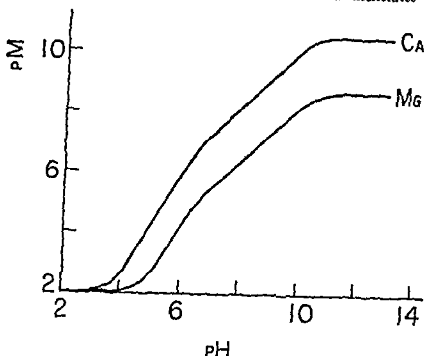
Since the first investigation of POPOVICI *et al* (1950), series studies of calcium homeostasis have been carried out SPENCER *et al* (1952) found that slow intravenous infusion of (the sodium salt of) tetracemin induced excess calciuria without lowering serum calcium levels This indicates promt replenishment of the complexed serum calcium by ionized calcium from the skeletal depots

According to this result, it is somewhat unlikely that serum calcium levels would have been lowered in our investigations, in which the tetracemin is given orally in single doses In fact, when tetracemin was infused intravenously at rates of 0 25 to 0.38 micromole/kg/minute for 82 hours, blood calcium levels rose by about 1 2 mg/100 ml in both goats and cows (TWARDOCK 1963) MAZZUOLI *et al* (1958) infused tetracemin intravenously into human subjects at a rate about 2-4 times higher than in TWARDOCK's experiments, and observed no ill effects in the course of a 6 hours infusion period

In the same investigation TWARDOCK made the peculiar observation that calcium tetracemin seemed to be more toxic than tetracemin to goats and cows when used for the purpose of removing Sr^{85} from the organism It is suggested that this might mean that tetracemin possesses toxicity other than that caused by its calcium binding property

In a series of reports on experimental and therapeutic uses of calcium tetracemin, such toxic effects are not mentioned

Further experiments on calcium metabolism have been carried out with tetracemin by several workers, whose results have been briefly reviewed by RUBIN (1963) Among the most interesting investigations for veterinary medicine are those of PAYNE (1964), who tested the maximum rate at which cows could replace their blood calcium to prevent hypocalcaemia Similarly, PAYNE & SANSOM (1963) tested the susceptibility of various groups of rats to hypocalcaemia Tetracemin has been employed



Relation of metal binding and hydrogenion concentration (MARTELL & CALVIN 1952 Cited by RUBIN & LINDENBLAD 1956)

Increasing concentrations of hydrogen ions will result in increasing dissociation of bound metal (see figure above) The concentration of calcium ions in equilibrium with tetracemin is expressed as a numerically positive pM function by conversion to the logarithm of the resiprocal of its concentration This is an analogous relation to the description of the hydrogenion concentration expressed by the pH value

MØLLER (1958) cites log K for some ions of biological and toxicological interest *Thus for the monovalent ions Na and Li, log K is 1 7 and* 2 8, respectively, and for some divalent ions it is Ba, 7 8, Mg, 8 7, Ca, 10 6, Fe^{++}, 14 8, Cd, 16 5, Pb, 18 2, Cu, 18 4 For trivalent ions it is Cr, 26 and Fe^{+++}, 25

This property of tetracemin provides a basis for its extensive use in experimental biology The first to realise this fact was POPOVICI *et al* (1950) *They found that in the physiological pH range of* human and rabbit serum the complex of calcium ion with tetracemin forms stoichiometrically in vitro and in vivo, and that under the same conditions tetracemin combines with calcium ion preferentially over magnesium

Metals higher in scale of binding ability have the ability to displace more weakly bound metals from chelate combination (RUBIN & LIN-

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for the purpose of determining absorbability of different calcium preparations from the intestine After lowering serum calcium with a relatively large intraperitoneal dose of tetracemin, the preparations are given orally to see at what rate the serum calcium is restored to normal (RUBIN *et al* 1960) Further, tetracemin has been used to test tolerance towards hypo calcaemia in different forms of rickets and to study renal clearance of calcium (RUBIN 1963)

Intestinal absorption of tetracemin and ions chelated by tetracemin is negligible In different species 4 % to a maximum of 18 % was absorbed by 24 hours after oral administration (FOREMAN & TRUJILLO 1954, PROESCHER 1951)

Sodium salts of tetracemin have been tolerated by rats in single oral doses as high as 4 g/kg (KRUM & FELLERS 1952) In experiments on absorption and metabolism of radioactive iron, tetracemin was given in amounts of 100 mg/kg for 7 days without any symptoms of hypocalcaemia Iron absorption was reduced, but excretion of radioiron in the urine was increased, apparently as a consequence of some absorption of tetracemin as such or of chelated iron (LARSEN *et al* 1960 a & b)

Tetracemin renders intestinal calcium unavailable to the organism (NICOLAYSEN & EEG LARSEN 1956) It will, as stated by THOMAS *et al* (1954), establish a nonabsorbable calcium reservoir in the intestinal tract This reservoir in the intestine competes by exchange across the intestinal membrane for the calcium in the blood thereby lowering urinary excretion and bone uptake, when no more calcium is available from the bone structure, the organism will be brought to a state of calcium deficiency

Since, however, the rate at which calcium ions under normal conditions are secreted into the intestine is fairly constant and slow (CRAMER) 1963), it is unlikely that amounts of calcium ions should leave circulation sufficient to contribute to hypocalcaemia after single oral doses of tetracemin Hypocalcaemia is also unlikely to occur, because of the negligible amount of tetracemin absorbed (FOREMAN 1953)

EFFECTS OF CALCIUM BINDING SUBSTANCES ON INTESTINAL ABSORPTION OF DRUGS

The reduction of toxic effects and lowering of blood concentrations of drugs

In the intact animal calcium binding substances (Ca b s) reduce the effect of drugs that they do not precipitate or form complexes with

Thus DYBING & SØGNEN (1960) found the toxic effect of red squill to be reduced when given orally with sodium oxalate, sodium fluoride or tetracemin This protecting effect of Ca b s on toxicity of red squill was not seen when the substances were given simultaneously parenterally, or the one by mouth and the other parenterally Ca b s were therefore believed to reduce the rate of intestinal absorption of red squill

Further experiments with intact rats and rabbits (SØGNEN 1961 & 1965 a) showed that the presence of Ca b s in the gastrointestinal tract reduced the effect of orally administered drugs Thus lethal oral doses of barbiturates and strychnine leave the animals unaffected or only slightly influenced when given concomitantly with Ca b s Under similar experimental conditions the blood levels of barbiturates, sulphanilamide, and alcohol were found to be lowered It was demonstrated that in the intact animal the effect of Ca b s on intestinal absorption was not due to difference in osmotic pressure in the solutions given to experimental and control animals, and since sulphanilamide administered in acid and alkaline solutions gave identical plasma concentrations of the drug, pH alterations caused by the Ca b s might be left out of account Premedication of Ca b s 7 days before the administration of sulphanilamide did not result in reduced blood concentration of this drug The effects of Ca b s on intestinal absorption were thus seen to be reversible Histological examinations at different times after administration of Ca b s did not reveal histological changes in the intestinal tract (ERICHSEN & SØGNEN unpublished)

Renal excretion

The lowering of the blood concentration of drugs in the experimental animals could be due to an increased renal excretion caused by Ca b s Experiments carried out to examine the effects of oral and intraperitoneal administration of Ca b s on sulphanilamide excretion and urine volume did, however, reveal that none of the Ca b s increased renal excretion of sulphanilamide It was further shown that those Ca b s, which were poorly

absorbed, reduced urine volume after oral administrations whereas sodium fluoride, which is absorbed to a considerable extent (ZIPKIN & LIKINS 1957), increased urine volume

After the intraperitoneal coadministration of sulphanilamide and Ca b s it was seen that sodium fluoride and sodium oxalate increased urine volume markedly, but sulphanilamide excretion was reduced The results justify the suggestion that renal sulphanilamide and water transport might be independent of each other (SØGNEN 1964 b)

Absorption from isolated loops in situ

In experiments with isolated intestinal loops in situ (SØGNEN 1965 c) it was found that though net transport of water from the intestine was inhibited, the effect on sulphanilamide absorption is questionable and the results depend on the experimental conditions At any rate the minor reduction of sulphanilamide absorption demonstrated, e g in experiments on colon, cannot be responsible for the marked reduction of the blood concentration seen in experiments with intact animals

Secretion, stomach emptying, and intestinal transit

Further investigations with sulphanilamide as the absorbable substance and phenol red as an unabsorbed marker (SØGNEN 1965 d) revealed that the reduced gastrointestinal absorption of drugs in presence of Ca b s is mainly due to a marked decrease in the rate of gastric emptying and probably also partly to an increased rate of passage through the small intestine

The retarded rate of gastric emptying is caused by a considerable increase in gastric secretion which begins rapidly after oral administration of Ca b s The volume of gastric fluid increases more rapidly as a result of the secretion than gastric contents can be removed from the stomach to the intestine In other words the dilution of the initial solution of test substances implies that less of the substances leave the stomach per unit of time And, accordingly, less of the substance to be absorbed (in this case sulphanilamide) is exposed to the absorbing surface of the intestinal epithelium

Absorption from perfused intestinal loops in situ

In the experiments with isolated loops in situ (SØGNEN 1965 c) it was found that Ca-b s caused fluid to be transported into the loop by secretion This involves a dilution of the initial concentration of the substances

whose absorption is to be studied, and does also alter the absorbing area and raise the intralumenal pressure in the loop Using SHEFF & SMYTH s method (1955), the conditions will be more comparable in the experimental and control animals Employing this method the small intestine is perfused from a reservoir containing a relatively large volume of fluid (30 50 ml) Pressure and dilution differences due to varying rates of water absorption are therefore, of no practical importance Under such experimental conditions (SØGNEN 1965 *e*) it was shown that though water and to some extent glucose transport are affected by Ca b s, sulphanilamide transport is practically unaffected by these compounds At concentrations of Ca b s which cause morphological changes the sulphanilamide transport is, however, slightly affected

It was found that the rate of disappearance of sulphanilamide from the intestinal lumen was somewhat decreased when glucose in the perfusion fluid was replaced with xylose which does not support water transport Since, however, sulphanilamide transport seems to be independent of water transport in the experiments with Ca b s, no explanation could be given for this observation

Transfer in vitro

In none of the experiments referred to above was it possible to study the transepithelial translocation alone without considering alterations of the blood circulation and other factors which may vary in situ The transport in vitro of glucose and sulphanilamide was therefore studied by WILSON & WISEMAN s method (1954) both in calciumfree Krebs-Ringer s solution alone and with Ca b s (SØGNEN 1965 *f*) It was shown that sulphanilamide is passively transported in everted loops of small intestine where conditions are suitable for the active transport of glucose Glucose transfer was reduced and sulphanilamide transfer was unaltered in the absence of calcium from the incubation fluid

A simultaneous reduction of both water and sulphanilamide transport was seen at concentrations of Ca b s causing profound morphological changes in the preparations

Immersion of the intestinal loop in solutions of Ca b s for 30 *seconds* prior to incubation revealed reduction in glucose transport which in most cases correlated with the morphological changes caused by the Ca b s

Glucose utilization in vitro

Finally, experiments were carried out to examine the glucose utilization in everted loops of small intestine in the absence of calcium and in the

presence of Ca b s (*SØGNEN 1965* g) The experiments indicated that Ca b s, which have been shown previously to influence water transport (SØGNEN 1965 c & e), also have a certain *inhibitory effect on the* utilization of glucose in vitro Since, however, the water transport is only partly dependent on glucose utilization (SMYTH 1962), and since the glucose utilization is not totally inhibited by the Ca b s it is unlikely that the altered water transport is caused by this inhibition alone The observations made *by* DUMONT *et al (1960) that water transport* depends on the concentration of calcium are referred to in the introduction to this survey The altered water transport in the intestine and the increased gastric secretion may, however, be caused by some less specific properties of Ca b s This is discussed elsewhere more extensively (SØGNEN 1965 d)

Discussion

The finding that Ca b s reduce the absorption of several drugs in the intact animal is supported by the results of GRASBECK & NYBERG (1958) and GRASBECK *et al* (1959) who reported tetracemin to depress the *absorption of vitamin* B_{12} *in man and calcium to raise the reduced* absorption of vitamin B_{12} in cases of steatorrhoea and pernicious anaemia ABELS *et al* (1959), however, saw no reduction in vitamin B_{12} absorption by gastrectomized rats when the vitamin was given together with gastric juice from the rat and tetracemin doses of the same order as in our work *These discrepancies are explained in our investigation where it is shown* that alterations in stomach emptying is the prime cause for the absorption inhibiting action of Ca b s

On the other hand the observation that Ca b s reduce intestinal absorption of drugs is apparently contradicted by the finding that tetracemin increases the absorption of heparin and heparinoid substances after oral coadministration to rats WINDSOR & CRONHEIM (1961) The explanation for this apparent contradiction must be sought in the difference in physicochemical properties of the drugs employed in the investigations to be compared

OVERTON (1902), HÖBER & HÖBER (1937) SCHANKER (1959) and HOGBEN *et al* (1959) have contributed to the finding that drugs cross cellular boundaries in accordance with their fat solubility Most drugs are weak electrolytes present in the body fluids as ionized and nonionized molecules (BRODIE 1964) Only the latter are lipid soluble, the ionized molecules not penetrating at an appreciable rate Substances with a pKa value between 2 and 9 are well absorbed but strong acids and bases are not (SCHANKER *et al* 1958) Transepithelial translocation of

drugs in the gastrointestinal tract thus depends largely on the lipid solubility of the nonionized molecules and on the proportion of drug present in this form, i e the degree of ionization (BRODIE 1964)

The substances employed in our investigations are all more or less undissociated at body pH, and the undissociated fraction is to some extent fat soluble, and thus passes through biological membranes at a rate independent of molecular size

The substances used by WINDSOR & CRONHEIM being fat insoluble, macromolecular acids do not normally pass through biological membranes

Since WINDSOR & CRONHEIM have used higher doses of tetracemin in their experiments, they must have got the same retardation of gastric emptying as seen in our experiments (SØGNEN 1965 d) The heparin compounds have, therefore, passed through the acid stomach very slowly LOOMIS (1959) has shown that small, but significant amounts of heparin are absorbed when the pH of the intestinal lumen is reduced to 1 or 2, and he suggested that sufficient amounts of nonionized heparin might be present to enter the epithelial cells

Another explanation on the increased heparin absorption demonstrated by WINDSOR & CRONHEIM is presented by SCHANKER & JOHNSON (1961) Their in situ experiments with isolated loops of rat small intestine demonstrated that the absorption of lipid insoluble compounds is enhanced, if tetracemin is present in the intestinal lumen The pH was maintained at 7 4 in these experiments, and the compounds tested were strong bases, neutral compounds, and strong acids which are not absorbed when administered alone Finding that inulin passed from the blood stream into the gut at a higher rate when tetracemin was present in the lumen, they suggested that the blood intestinal boundary had become more permeable as far as fat insoluble compounds were concerned They further suggested that tetracemin may act by increasing the size of the membrane «pores» or by widening the spaces between the epithelial cells through the removal of calcium ions

If this theory were right, it would be in nice agreement with the findings of WHITTEMBURY

of calcium from the

Ca sucrose (molecular

permeable enters quite readily However, raffinose, whose radius is 6 0 Å, is still excluded from the cell

Accordingly, the effect of tetracemin suggested by SCHANKER & JOHNSON would only enhance the transport of substances with limited molecular size

The pore radius of most cells — mucosal cells of rat small intestine included — is about 4 Å (SOLOMON 1960).

presence of Ca-b s (SØGNEN 1965 g) The experiments indicated that Ca-b s, which have been shown previously to influence water transport (SØGNEN 1965 c & e), also have a certain inhibitory effect on the utilization of glucose in vitro Since, however, the water transport is only partly dependent on glucose utilization (SMYTH 1962), and since the glucose utilization is not totally inhibited by the Ca b s it is unlikely that the altered water transport is caused by this inhibition alone The observations made by DUMONT *et al* (1960) that water transport depends on the concentration of calcium are referred to in the introduction to this survey The altered water transport in the intestine and the increased gastric secretion may, however, be caused by some less specific properties of Ca-b s This is discussed elsewhere more extensively (SØGNEN 1965 d)

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which might be of interest in transport mechanisms, cannot be chelated without causing structural changes due to binding of calcium also in the protein structures

In the intact rat, however, the Ca b s in amounts up to 400 mg/kg, do not cause morphological changes which can be revealed by usual histo logical methods (ERICHSEN & SØGNEN unpublished)

The increased secretion caused by the Ca b s may be due specifically to calcium binding, or it may be due to other nonspecific properties of the substances This increased secretion will lead to retarded gastric emptying, and, accordingly, to reduced intestinal absorption of most absorbable drugs The fact that net water transport from the intestinal lumen is reduced does not seem to influence transepithelial translocation such as that of for example sulphanilamide This might indicate that sulphanilamide passes through the intestinal mucosa independently to the simultaneous water transport

Summary

A survey is given of literature dealing with the effects of calcium on glucose and water absorption Some properties of calcium binding sub stances relevant to this problem are presented

It was assumed that drugs might depend on water absorption for their transfer throught the intestinal mucosa This assumption originated from the observation that in the intact animal calcium binding substances reduce toxic effects and lower the blood concentrations of several drugs

The experiments carried out to investigate the mechanism behind this observation, however, revealed that reduced gastrointestinal absorption of drugs in the presence of calcium binding substances is mainly due to a retarded rate of gastric emptying Though glucose and water transport was found to be reduced in the presence of calcium binding substances, it was found that sulphanilamide passes the intestinal mucosa *apparently* independently to the transport of water

The implications of these findings are discussed in relation to the results of other workers

The undissociated sulphanilamide is somewhat fat soluble, and will thus probably pass biological membranes independently of alterations in pore size and intercellular spaces It might, therefore, be reasonable to assume that be transport of this substance is unaffected or only slightly influenced by alterations of pore sizes

In our experiments, however, the Ca-b s caused a disintegration of the intestinal mucosa in vitro and in some experiments with isolated loops in situ (ERICHSEN & SØGNEN unpublished) This may be the case also in the experiments of SCHANKER & JOHNSON It should therefore be justifiable to suggest that the adsorption of macromolecular, watersoluble substances is facilitated (made possible) when the mucosal epithelium is altered, whereas the absorption of fat soluble compounds such as sulphanilamide is slightly decreased or uninfluenced under such circumstances

Disintegration of intestinal mucosa by tetracemin in vitro has previously been reported by HANSEN (1959) Similarly, KELEYI & KASZA (1958) have found tetracemin to cause local oedema when injected subcutaneously According to HAMM (1955) the effects of Ca-b s on proteins are due to removal of the Ca^{++} and Mg^{++} from binding sites between the peptide chains in protein molecules of the various tissues

In a discussion following a paper read by SOLOMON (1960) dealing with the effect of calcium on pore radius, MAIZELS (1960) referred to some experiments (CLOWES 1916) which showed that in an emulsion of oil and water the state of dispersion depended on the proportion of calcium and sodium With a preponderance of calcium chloride there would be a continuous oil phase, with a preponderance of sodium chloride a continuous water phase would result He posed a question whether more complex systems would be more specifically affected by calcium than would simple systems

HODGKIN & KATZ (1949) cited by KEYNES made an observation which might be relevant to this question They found that if the inside of a squid axon is squeezed out in isotonic KCl it will stay as a well defined rod of jelly for hours, whereas the presence of only 4 mM calcium in the medium would make the rod dissappear in a few minutes This was found to be entirely specific for calcium

Extensive discussions concerning the role of calcium in membrane tran sport and metabolism are recently given by KLEINZELLER & KOTYK (1960) and WASSERMAN (1963)

Obviously, calcium exerts a stabilizing effect on the structure of membranes, but its function in these systems is not fully understood

As far as the intestinal mucous membrane is concerned, it seems to be difficult to establish calcium-free conditions in vitro by means of Ca b s, apparently because the transportable and loosely bound calcium ions,

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Studies on biogenic amines and reserpine induced block of the diuretic action of hydrochlorothiazide and theophylline in the chicken

BY

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A summary is given based upon the present (I) and four earlier works by the author.

Nechay & Sanner 1961 a (II),
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Contents

Introduction

It has been shown that reserpine blocks the diuretic effect of theophylline and hydrochlorothiazide (NECHAY & SANNER 1961 b)

In these experiments no specific measures were taken to ensure a large tubular urine flow Since failure of diuretic action could conceivably be due to reduced tubular flow isotonic mannitol was used in the present experiments where results on theophylline diuresis will be presented

A restoration of reactivity to sympathetic stimuli in reserpinized animals has been achieved by infusion of catecholamines (BURN & RAND 1958 1960 ROSELL & SEDVALL 1961 MIRKIN & v EULER 1963) This in all probability was due to a repletion of catecholamine stores

This suggested an attempt to refill the catecholamine stores of the kidneys in reserpinized birds by giving a catecholamine before the main diuresis experiment and thus restitute the diuretic effect of theophylline Dopamine was chosen for its minor blood pressure effects GREEN & SIM (1961) also showed that dopamine itself has diuretic effects in the rat During the completion of this work some effects of dopamine on renal activity has been reported in the dog (McNAY et al 1963) and in man (McDONALD et al 1964)

By using chickens it is possible to increase the dopamine concentration in the renal tubules unilaterally since the birds have a renal portal circulation (SPERBER 1949) and part of the renal tubules are provided with blood from the leg vein

A. EXPERIMENTAL ANIMALS

Rhode Island Red chickens weighing 2.0—3.4 kg were used. The birds were kept on commercial chicken food and fresh water ad libitum. Generally the birds were fasted during the night preceeding the experiment. Not more than two experiments were done on each bird. At least one week resting time was allowed between the experiments and reserpine was used last. The reserpine pretreated birds used in this study were injected with 1 mg/kg reserpine into the pectoral muscle 17—19 hours before the main experiment. The birds were unanaesthetized during the diuresis experiments and the glomerular filtration rate experiments.

B. BLOOD PRESSURE MEASUREMENTS

The mean arterial blood pressure (MAP) was recorded through a polyethylene catheter inserted into the ischiadic artery. A pressure transducer (EMT 490 A, Elema Schonander, Solna, Sweden) and an electromanometer were used. The pressure was registered by a Varian G 10 graphic recorder. In these experiments the birds were under light pentobarbital anaesthesia.

C. GLOMERULAR FILTRATION RATE

The renal clearance of polyethylene glycol (PEG) average molecular weight 4000 was used as a measure of glomerular filtration rate (GFR) according to HYDÉN & KNUTSON (1959), HYDÉN (1955).

After a priming injection of 5 ml PEG solution (125 mg/ml) into the wing vein a continuous infusion of 0.25 ml/min was given throughout. At least 20 minutes equilibration time was allowed before measurements were begun. Then blood samples were drawn into heparinized centrifuge tubes at the mid point of each urine collection period. The blood was immediately centrifuged and 1 ml of the clear plasma was pipetted off. Protein and other interfering substances were removed according to SOMOGYI (1930) as modified by SMITH (1956).

D ELECTROLYTES

Chloride was analysed electrometrically (COTLOVE et al 1958) Sodium and potassium were analysed by a direct reading flame photometer (EPPENDORF)

E URINE COLLECTION

Campbell's (CAMPBELL 1960, CAMPBELL & SJOBERG 1960) mechanised modification of the original SPERBER (1948, 1949) technique was used for urine collection

F DRUGS AND SOLUTIONS

All doses refer to the salts or complexes

Polyethylene glycol Polyethylene glycol (PEG) with an average molecular weight of 4000 was used, at a concentration of 12 5 per cent w/v in 0 72 per cent sodium chloride

Mannitol A 5 5 per cent w/v mannitol solution was made in distilled water (d mannitol Difco Certified)

Reserpine Commercial reserpine (Serpasil®, Ciba Basle and Serpedin®, Pharmacia, Uppsala) was provided in ampouls containing 2 5 mg/ml and used as such

Theophylline A solution of 8 mg/ml in 0 9 per cent sodium chloride was prepared each day

Dopamine Dopamine (3 hydroxytyramine hydrochloride, Hoffman La Roche, Basle), in powder form, was dissolved in 0 9 per cent sodium chloride to make 2 5 mg/ml Dopamine solutions were prepared just before use

Serotonin Serotonin creatinine sulphate (*Sandoz, Basle*) provided in ampouls containing 10 mg/ml of the complex was dissolved in 0 9 per cent sodium chloride to make 250—500 µg/ml

Catron JB 516, 1-phenyl 2 hydrazinopropane hydrochloride (Catron® Lakeside Laboratories, Milwaukee, Wisconsin) was prepared just before use in 0 9 per cent sodium chloride to make 10 mg/ml

Phenol red 1 0 mg/ml in 0 9 per cent sodium chloride

G NET EXCRETION

The term net *excretion/hour refers to the following calculation* The control excretion calculated as excretion/hour in the last 10 minute period immediately preceeding the diuretic injection is subtracted from the measured excretion during the following 60 minutes

II STATISTICS

The means and their standard errors were calculated for the net excretion/hour The means were compared and the significances were tested by Student's t test As some of the distributions were skew, all the data were transformed to logarithmic form for tests of significance Nonetheless ordinary standard errors of the mean are shown in the tables to give an idea of the variability

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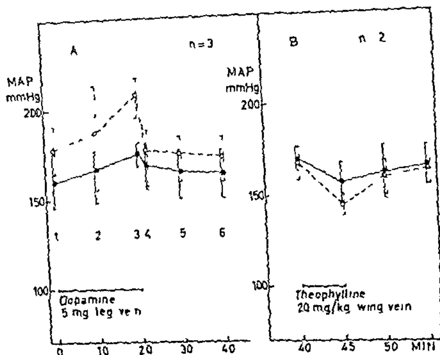


Fig 1 A
Mean arterial pressure (MAP) in reserpine pretreated and normal birds given dopamine into the leg ve n (5 mg over 20 minutes) *Dotted lines* , reserpine pretreated birds solid lines —— normal birds Vertical bars indicate the range (3 b r ls each)

F g 1 B
The MAP measured during and after theophylline injection into the wing vein (20 mg/kg over 5 minutes) Theophylline was given 20 minutes after the dopamine injection was ended to 2 of the 3 birds earlier given dopamine Dotted lines reserpine pretreated b rds sol d lines —— normal birds Vertical bars indicate the range 2 b rds each)

III ACTION OF DIURETICS NORMAL AND RESERPINIZED BIRDS

1 Normal birds

A Mannitol infusions

Control experiments in 3 birds were run where isotonic mannitol was infused into the wing vein at 0 25 ml/min after a priming in jection of 20 ml Thirty minutes infusion was allowed for equilibration Thereafter the excretion was measured for the following 60 minutes (table 1)

Mannitol infusion 0 25 ml/min, was also given to a series of 14 non reserpinized birds after a priming injection of 20 ml (M, table 2)

Results

I BLOOD PRESSURE MEASUREMENTS

Three reserpine pretreated birds and three untreated birds were used Dopamine was infused into one leg vein (5 mg given over 20 minutes) The mean arterial blood pressure (MAP) was read at the following points on the curve (Fig 1 A) 1 Before the dopamine infusion (time zero) 2 At the middle of the dopamine infusion 3 At the end of the dopamine infusion 4 About 2 minutes after the dopamine infusion was stopped Pressures were also determined at 10 and 20 minutes after the dopamine infusion was stopped (5 & 6)

From figure 1 A it is seen that dopamine infusion into the leg vein produced a moderate elevation of the MAP This effect was however shortlasting Comparison of the starting values in Fig 1 A also shows that the dose of reserpine used in this study did not decrease the MAP as measured by this method The values obtained are high but are within the normal range for chickens (STUBEL 1910)

The MAP was also read in 2 of the 3 chickens during theophylline injection (20 mg/kg into the wing vein over 5 minutes) (Fig 1 B) Theophylline was given 20 minutes after the dopamine infusion was ended Here a slight depression of the MAP was observed in both reserpine pretreated and normal birds (2 birds in each group) This confirmed earlier findings in non reserpinized birds (NECHAY & SANNER 1961 a)

II GLOMERULAR FILTRATION RATE

The GFR in two consecutive 20 minute periods were measured and averaged In 14 normal birds the GFR was 5 22 ± 0 33 ml min (2 21 ± ± 0 11 ml/kg/min) as compared to 22 reserpine pretreated birds where the GFR was 4 93 ± 0 24 ml/min (2 23 ± 0 12 ml/kg/min) The figures indicate the means with their standard errors Thus 1 mg/kg reserpine given 17—19 hours before did not decrease the glomerular filtration rate

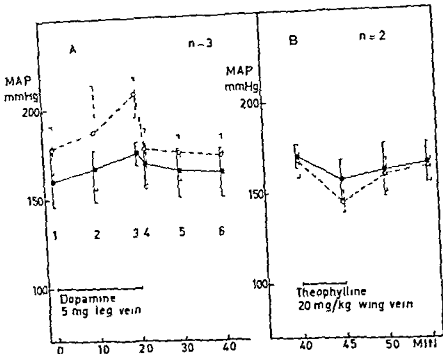


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Fig 1 B
The MAP measured during and after theophylline injection into the wing vein ... opamine ... cate the

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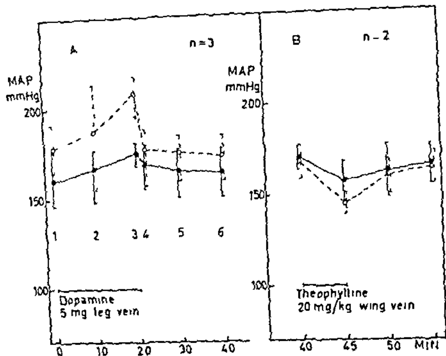


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Fig 1 B

The MAP measured during and after theophylline injection into the wing vein [illegible] given 20 minutes after the dopamine [illegible] given dopamine Dotted lines [illegible] ormal birds Vertical bars indicate the [illegible]

III ACTION OF DIURETICS NORMAL AND RESERPINIZED BIRDS

1 *Normal birds*

1 *Mannitol infusions*

Control experiments in 3 birds were run where isotonic mannitol was infused into the wing vein at 0 25 ml/min after a priming injection of 20 ml *Thirty* minutes infusion was allowed for equilibration Thereafter the excretion was measured for the following 60 minutes (table 1)

Mannitol infusion 0 25 ml/min, was also given to a series of 14 non reserpinized birds after a priming injection of 20 ml (M, table 2)

Results

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Three reserpine pretreated birds and three untreated birds were used Dopamine was infused into one leg vein (5 mg given over 20 minutes) The mean arterial blood pressure (MAP) was read at the following points on the curve (Fig 1 A) 1 Before the dopamine infusion (time zero) 2 At the middle of the dopamine infusion 3 At the end of the dopamine infusion 4 About 2 minutes after the dopamine infusion was stopped Pressures were also determined at 10 and 20 minutes after the dopamine infusion was stopped (5 & 6)

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Table 2

Parameter	Non reserp nized b rds			Reserpinized I irds			
	Non injected (M_a)	During manni tol infusion wing vein (M_c)	After dopamine into one leg vein (M_e)	Non injected (M_b)	During manni tol infusion wing vein (M_d)	After dopamine into one leg vein (M_f)	After dopamine leg vein during mannitol (M_g)
Cl^-, µeq/hr	83 ± 33	350 ± 120	137 + 41	54 ± 18	92 ± 32	74 ± 13	69 ± 18
Na^+, µeq/hr	92 ± 9	440 + 150	174 ± 31	103 ± 10	97 ± 13	91 ± 15	150 ± 37
K^+, µeq/hr	69 ± 19	134 ± 21	118 ± 42	206 ± 68	77 ± 17	88 ± 24	116 ± 21
Urine, ml/hr	3 6 ± 1 0	20 4 ± 3 6	6 1 ± 1 9	2 1 ± 0 4	11 8 ± 1 0	3 1 ± 0 7	11 4 ± 1 0
Number of birds	5	14	5	5	12	5	7

Excretion values calculated as excretion/hour in the last 10 minute period immediately preceeding theophylline injection (both kidneys together)

Table 1

Influence of mannitol infusion

Experimental animal	Cl^-, µeq /hr	Na^+, µeq /hr	K^+, µeq /hr	Urine ml/hr
Non reserpinized birds (n=3)	195 (152—230)	272 (170—453)	115 (82—151)	15 7 (10 4—21 8)
Reserpinized birds (n 3)	85 (52—143)	126 (62—243)	126 (52—193)	9 4 (7 8—10 0)

Chloride sodium potassium and water excretion in reserpinized and non reserpinized birds (both kidneys together) After a priming injection of 20 ml mannitol into one wing vein 30 minutes constant infusion at 0 25 ml/min were allowed for equilibra tion *before the excretion* values were taken The excretion was measured *for the* following 60 minutes under continuous mannitol infusion

At least 20 minutes were allowed for equilibration Five control animals were not infused (M_a table 2) The values refer to the last 10 minutes before the subsequent injection of theophylline There was an increase of electrolyte excretion and urine flow by mannitol

B Theophylline injections

Theophylline was given at a dose of 20 mg/kg into the wing vein in a group of 5 non reserpinized birds and the excretion over one hour *was measured The net excretion/hour was calculated (M_a table 3)*

C Theophylline in conjunction with continuous mannitol infusion

When 20 mg/kg theophylline was given to non reserpinized birds as a single wing vein injection lasting 5 minutes during a continuous mannitol infusion (0 25 ml/min) there was a large increase in diuresis for sodium chloride and urine flow but only a moderate increase in potassium excretion (14 birds M_c table 3) The values for net excre tion/hour were compared to the corresponding ones when theophylline was given to 5 non reserpinized birds where mannitol was *not given* (M_a vs M table 3) The table shows that isotonic mannitol infusion as such did not produce any *increase of the net water and electrolyte* excretion in normal birds after theophylline If anything in the manni tol experiments in normal birds theophylline produced a somewhat smaller diuretic effect A statistical analysis *however did not show* any significant difference between the two groups Probably also too *much had been subtracted in the mannitol experiments by using some* pre periods where a fall in excretion rate was still going on

Table 2

Parameter	Non-reserpinized birds: Non injected (M_a)	Non-reserpinized birds: During mannitol infusion, wing vein (M_c)	Non-reserpinized birds: After dopamine into one leg vein (M_e)	Reserpinized birds: Non injected (M_b)	Reserpinized birds: During mannitol infusion, wing vein (M_d)	Reserpinized birds: After dopamine into one leg vein (M_f)	Reserpinized birds: After dopamine leg vein during mannitol (M_g)
Cl^-, μeq/hr.	83 ± 33	350 ± 120	137 ± 41	54 ± 18	92 ± 32	74 ± 13	69 ± 18
Na^+, μeq/hr	92 ± 9	140 ± 150	174 ± 31	103 ± 10	97 ± 13	91 ± 15	150 ± 37
K^+, μeq/hr	69 ± 19	134 ± 21	118 ± 42	206 ± 68	77 ± 17	88 ± 24	116 ± 21
Urine, ml/hr	3.6 ± 1.0	20.4 ± 3.6	6.1 ± 1.9	2.1 ± 0.4	11.8 ± 1.0	3.1 ± 0.7	11.4 ± 1.0
Number of birds	5	14	5	5	12	5	7

Excretion values calculated as excretion/hour in the last 10 minute period immediately preceeding theophylline injection (both kidneys together)

Table 3

Parameter	Non reserpinized birds		Reserpinized birds		Reliability of difference between means (log x) p values	
	Theophylline (M_a)	Theophylline during mannitol (M_c)	Theophylline (M_b)	Theophylline during mannitol (M_d)		
Cl^-, meq/hr	4 7 ± 1 3	2 90 ± 0 51	0 059 ± 0 037	1 28 ± 0 37	M_a-M_b < 0 01 M_c-M_d < 0 01	M_d-M_b < 0 01 M_a-M_c > 0 10
Na^+, meq/hr	4 1 ± 1 2	2 41 ± 0 30	0 457 ± 0 19	1 25 ± 0 27	M_a-M_b < 0 05 M_c-M_d < 0 01	M_d-M_b < 0 05 M_a-M_c > 0 10
K^+, meq/hr	0 45 ± 0 11	0 181 ± 0 043	0 177 ± 0 037	0 177 ± 0 039	M_a-M_b < 0 05 M_c-M_d > 0 10	M_d-M_b > 0 10 M_a-M_c < 0 05
Urine ml/hr	63 ± 19	41 0 ± 4 6	2 0 ± 1 2	20 0 ± 5 4	M_a-M_b < 0 01 M_c-M_d < 0 05	M_d-M_b < 0 02 M_a-M_c > 0 10
Number of birds	5	14	5	12		

Influence of mannitol infusion on net excretion/hour after injection of 20 mg/kg theophylline in reserpinized and non reserpinized birds compared to birds not treated with mannitol (both kidneys together)

2 *Reserpinized birds*

A *Mannitol infusions*

Control experiments were run in 3 reserpinized birds where isotonic mannitol was infused into the wing vein at 0 25 ml/min after a priming injection of 20 ml Thirty minutes infusion was allowed for equilibration An antidiuretic effect of reserpine could be seen when the excretion measured over one hour was compared to that obtained in non reserpinized birds treated in the same way (table 1)

Isotonic mannitol solution was also given to a group of 12 reserpine pretreated birds after a priming injection of 20 ml At least 20 minutes infusion at 0 25 ml/min was allowed for equilibration (M_d table 2) The values refer to the last 10 minutes before the subsequent injection of theophylline Chloride excretion was somewhat larger but sodium excretion was similar compared to reserpinized birds not given mannitol (M_d vs M_b table 2) Potassium excretion however, was smaller in the mannitol group Water excretion was much higher than in reserpinized birds not receiving mannitol but did not reach values as high as in normal birds given mannitol (M_d vs M_c table 2)

B *Theophylline injections*

Theophylline was given at a dose of 20 mg/kg into the wing vein in a group of 5 reserpine pretreated birds and the net excretion/hour was calculated (Table 3 M_b vs M_a) Earlier results were confirmed (NECHAY & SANNER 1961 b) the diuretic effect of theophylline being virtually blocked Both electrolyte—and water excretion were depressed Chloride and water excretion were more depressed than sodium excretion Potassium excretion was also only moderately depressed ($p < 0\ 05$)

C *Theophylline in conjunction with continuous mannitol infusion*

When 20 mg/kg theophylline was given as a single wing vein injection lasting 5 minutes during a continuous mannitol (0 25 ml/min) infusion to a group of 12 reserpine pretreated birds there was a greatly increased diuretic effect compared to that obtained in a group of 5 reserpinized birds where no mannitol was given (M_d vs M_b, table 3) A statistical evaluation showed a significant difference for chloride ($p < 0\ 01$) and urine flow ($p < 0\ 02$) For sodium excretion the significance was smaller ($p < 0\ 05$) and for potassium the excretion was identical with that of reserpinized birds which were not given mannitol

3 Normal and reserpinized birds

Theophylline in conjunction with continuous mannitol infusion

A comparison between the diuretic effect of theophylline in normal and reserpinized birds both groups given a continuous mannitol infusion of 0 25 ml/min was also performed (M_e vs M_1 table 3)

Statistical test showed a significant difference for chloride ($p<0\ 01$) and sodium ($p<0\ 01$) and urine excretion ($p<0\ 05$) but potassium excretion ($p>0\ 10$) was not significantly different in the two groups Thus mannitol infusion was not sufficient to reverse the reserpine block and produce a full diuretic response after theophylline in reserpinized birds

IV DOPAMINE REVERSAL OF RESERPINE BLOCK OF THEOPHYLLINE ACTION

1 Normal birds

A Unilateral dopamine injection (total excretion measured)

Dopamine at a dose of 5 mg was given into one leg vein as an injection lasting 20 minutes to a group of 5 non reserpinized birds Values refer to the total excretion for both kidneys They were taken in the last half of the 20 minute period following the dopamine injection and preceeding theophylline Table 2 M_e vs M_a shows a tendency towards increased electrolyte and urine excretion

B Theophylline after unilateral dopamine (total excretion measured)

In the same non reserpinized birds 20 mg/kg theophylline was injected into the wing vein 20 minutes after the dopamine injection was ended *The net excretion during the next hour for both kidneys together* is compared to the same in normal birds where no dopamine pretreatment had been given (M_a *table 3* vs M_e *table 4*) Potassium excretion was smaller in the dopamine group (difference probably significant $p<0\ 05$) but for chloride and sodium excretion and urine flow only a slight tendency for the excretion of the dopamine treated animals to be smaller was seen

C Unilateral action of theophylline after dopamine

(excretion measured for each kidney separately)

Fig 2 shows the data for each kidney separately in the same 5 experiments in *non reserpinized birds* The figure shows also the ratio

between the injected side and the noninjected side after a unilateral priming with dopamine before theophylline injection into the wing vein (mean of 5 birds)

The dopamine injection into the leg vein probably increased the homolateral electrolyte and urin excretion to some extent before theophylline

After theophylline the excretion rate was very little larger on the dopamine injected side compared to the noninjected side In some periods the excretion rate was even smaller on the dopamine injected side

The ratios between injected and non injected side were calculated and averaged

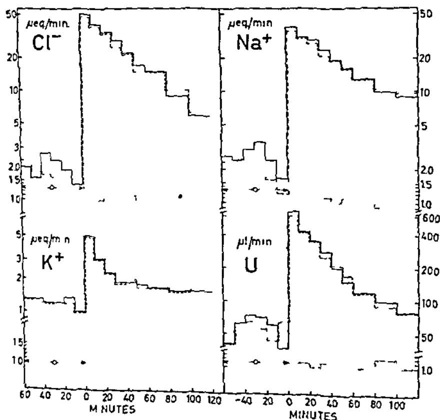


Fig 2

Normal birds primed with 5 mg dopamine given into one leg vein at (—40) to (—20) minutes Theophylline is given at (0—5) minutes into one wing vein Electrolyte and urine excretion rate for each kidney is given separately Solid lines ——, dopamine injected side dotted lines .... non injected side Ratios injected side to non injected side are indicated by — — — — — The average ratio over the preperiod indicated is shown by ← — —0— — → (5 birds)

In the prediuretic urine collection periods the excretion rate was small. Ratios were thus subject to great variations since a small deviation in excretion rate was reflected by large swings in excretion ratios. In the prediuretic period the ratio for each bird was therefore calculated between the excretion during the whole 60 minutes prediuretic period for each side. The average ratio for the 5 birds was greater than one for sodium, chloride and urine excretion but not for potassium where it was close to one.

After theophylline the mean ratios were calculated from the ratios for each urine collection period separately. Here the ratios were generally close to one.

This procedure has also been used in the following treatment of the data from reserpinized animals.

2. *Reserpinized birds*

A. Unilateral dopamine injection (total excretion measured)

Dopamine at a dose of 5 mg was injected into one leg vein over 20 minutes to a group of 5 reserpinized birds (M_f, table 2). Values refer to the total excretion for both kidneys. They were taken in the last half of the 20 minute period following the dopamine injection and preceding theophylline. This dopamine injection, if anything, produced a decreased potassium excretion which was high in another group of 5 reserpinized birds not given dopamine (table 2, M_f vs M_e). The high potassium excretion in reserpinized birds was in accordance with earlier findings (NECHAY & SANNER 1961 b).

Sodium and chloride excretion and urine flow were, however, similar in the two groups of birds.

B. Theophylline after unilateral dopamine (total excretion measured)

In the same 5 reserpinized birds 20 mg/kg theophylline was given as a single wing vein injection 20 minutes after the dopamine injection was ended. The net excretion/hour for both kidneys together was taken (M_f, table 4).

This injection procedure greatly increased the excretion of both electrolytes and urine, which was not significantly different from the ones obtained in a group of non reserpinized birds under otherwise identical conditions (M_e vs M_f, table 4).

A comparison between dopamine *plus* theophylline and theophylline alone given to reserpine pretreated birds (M_f, table 4 vs M_e, table 3)

Table 4

Parameter	Non reserpinized birds		Reserpinized birds			Reliability of difference between means (log x) *indicates values presented in table 3 p values		
	Dopamine and Theophylline (M_a)	Mannitol and Theophylline (M_e)	Dopamine and Theophylline (M_f)	Mannitol and Theophylline (M_d)	Mannitol, Dopamine and Theophylline (M_g)			
Cl^-, meq/hr	3.6 ± 1.2	2.90 ± 0.51	2.3 ± 1.5	1.28 ± 0.37	2.53 ± 0.51	M_a–M_f > 0.10 M_f–M_b* < 0.01	M_a*–M_e > 0.10 M_e–M_g > 0.10	M_g–M_d > 0.05 M_g–M_f > 0.10
Na^+, meq/hr	2.89 ± 0.85	2.41 ± 0.30	2.16 ± 0.93	1.25 ± 0.27	2.37 ± 0.39	M_a–M_f > 0.10 M_f–M_b* > 0.10	M_a*–M_e > 0.10 M_e–M_g > 0.10	M_g–M_d < 0.05 M_g–M_f > 0.10
K^+, meq/hr	0.197 ± 0.013	0.181 ± 0.013	0.244 ± 0.050	0.177 ± 0.039	0.297 ± 0.052	M_a–M_f > 0.10 M_f–M_b* > 0.10	M_a*–M_e < 0.05 M_e–M_g > 0.05	M_g–M_d > 0.05 M_g–M_f > 0.10
Urine, ml/hr	35 ± 11	41.0 ± 4.6	24 ± 15	20.0 ± 5.4	35.9 ± 6.1	M_a–M_f > 0.10 M_f–M_b* < 0.02	M_a*–M_e > 0.10 M_e–M_g > 0.10	M_g–M_d > 0.10 M_g–M_f > 0.10
Number of birds	5	14	5	12	7			

Influence of dopamine and mannitol on reserpine induced block of theophylline diuresis. All values are net excretion/hour for both kidneys together. An asterisk indicates values presented in table 3.

shows a significant increase in chloride and urine excretion in the dopamine group ($p < 0.01$ and $p < 0.02$, respectively). Sodium and potassium excretion were also greater *when dopamine was given before* theophylline, but here no statistically significant difference was obtained ($p > 0.10$) However, in those experiments where dopamine was given to reserpinized birds during mannitol infusion, it had a probably significant effect even on the natriuretic effect of theophylline (M_g vs M_d table 4, $p < 0.05$).

The dopamine effect persists for about one hour. When 5 mg dopamine had been unilaterally injected one hour before 20 mg/kg theophylline in 4 reserpinized birds, a diuretic response was obtained in three of them that was considerably greater than in reserpinized birds *not treated* with dopamine

C Unilateral action of theophylline after dopamine

(excretion measured for each kidney separately)

Fig 3 shows data for the two kidneys separately in the same 5 experiments in reserpinized birds The figure shows the excretion on the injected side and non-injected side separately and also the ratio between the injected side and non-injected side after a unilateral priming with dopamine before theophylline injection into the wing vein (mean of 5 birds).

The dopamine injection into one leg vein did not increase electrolyte- or urine excretion in the prediuretic period

However, after theophylline the excretion rate was much increased, and it should be noted that the excretion values on the dopamine injected side were larger

In the 60 minutes prediuretic urine collection period the average ratio between injected side and non-injected side was close to one for urine and potassium, but slightly above one for chloride and sodium

After theophylline injection, however, the ratios were considerably greater than one for both electrolyte- and urine excretion This was most apparent for chloride and urine

That this unilaterality cannot be due to chance for instance the kidneys on the injected side being larger than the control kidneys is concluded from the following If this were so any dissimilarities between the two kidneys should be apparent for both chloride and sodium This was not the case, chloride excretion was definitely more unilateral This suggests a renal effect by dopamine facilitating the theophylline effect mainly on chloride excretion

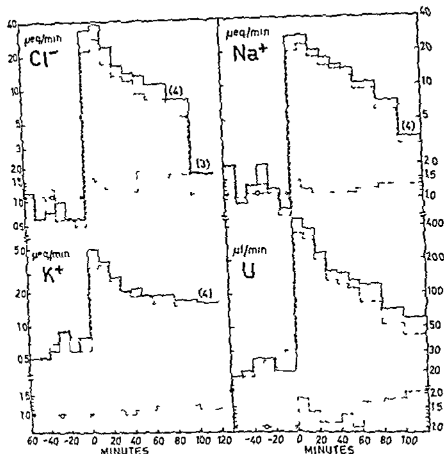


Fig 3

Reserpine pretreated birds primed with 5 mg dopamine given into one leg vein at (—40) to (—20) minutes Theophylline is given at (0—5) minutes into one wing vein Electrolyte and urine excretion rate for each kidney is given separately Solid lines —— dopamine injected side dotted lines ...... non injected ...
Ratios ...
ratio ...
in p ...

D Theophylline after dopamine mannitol

(total excretion measured)

In a group of 7 ...

infused systemicall ...

After a priming in ...

libration During this time 5 mg dopamine was injected over 20 minutes into one leg vein The dopamine injection was completed 20 minutes before theophylline 20 mg/kg was given into the wing vein as a

shows a significant increase in chloride and urine excretion in the dopamine group ($p<0.01$ and $p<0.02$, respectively) Sodium and potassium excretion were also greater when dopamine was given before theophylline but here no statistically significant difference was obtained ($p>0.10$) However, in those experiments where dopamine was given to reserpinized birds during mannitol infusion, it had a probably significant effect even *on the natriuretic effect of theophylline* (M_g vs M_d table 4, $p<0.05$)

The dopamine effect persists for about one hour When 5 mg dopamine had been unilaterally injected one hour before 20 mg/kg theophylline in 4 reserpinized birds, a diuretic response was obtained in three of them that was considerably greater than in reserpinized birds not treated with dopamine

C Unilateral action of theophylline after dopamine

(excretion measured for each kidney separately)

Fig 3 shows data for the two kidneys separately in the same 5 experiments in reserpinized birds The figure shows the excretion on the injected side and non injected side separately and also the ratio between *the injected side and non injected side after a unilateral priming* with dopamine before theophylline injection into the wing vein (mean of 5 birds)

The dopamine injection into one *leg* vein did not increase electrolyte or urine excretion in the prediuretic period

However after theophylline the excretion rate was much increased and it should be noted that the excretion values on the dopamine injected side were larger

In the 60 minutes prediuretic urine collection period the average ratio between injected side and non injected side was close to one for urine and potassium but slightly above one for chloride and sodium

After theophylline injection however the ratios were considerably greater than one for both electrolyte and urine excretion This was most apparent for chloride and urine

That this unilaterality cannot be due to chance for instance the kidneys *on the injected side* being larger than the control kidneys is concluded from the following If this were so any dissimilarities between the two kidneys should be apparent for both chloride and sodium This was not the case chloride excretion was definitely more unilateral This suggests a *renal effect by dopamine* facilitating the theophylline effect mainly on chloride excretion

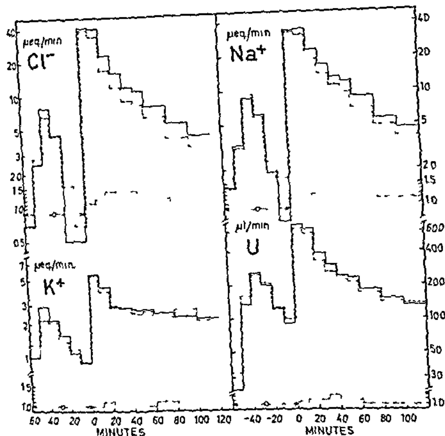


Fig. 6
...
... minutes theophylline 20 mg/kg was injected into the wing vein. Electrolyte and urine excretion rate for each kidney is given separately. Solid lines —— dopamine injected side, dotted lines ---- non injected side. Ratios injected side to non injected side are indicated by — — — — — — The average ratio over the preperiod indicated is shown by ← — —0— — → (7 birds)

did not reach values as high as in the reserpinized birds given dopamine but not mannitol (Fig. 3). Again there was a *tendency for* the chloride excretion to be more unilateral than the excretion of *sodium*.

V. TESTING OF BIRDS

All the birds primed with dopamine into one leg vein were tested with 0.2 mg/kg phenol *red into the same* leg vein. They all excreted the phenol red with a visible excess on the injected side.

single injection over 5 minutes. The net excretion in the hour following theophylline was calculated for both kidneys together and compared to the excretion values obtained in 14 normal birds given mannitol and theophylline only (M_c vs M_g, table 4). The excretion values were very similar in the two groups except for potassium which was insignificantly higher in the dopamine experiments in reserpinized birds. Thus dopamine injection increased the diuretic effect of theophylline in reserpine pretreated birds during mannitol infusion to the pre-reserpinization level.

When a comparison between M_g and M_t in table 4 was made a greater excretion was observed where dopamine had been given. The difference was probably significant for sodium ($p<0.05$) but not for chloride, potassium and urine.

However, a comparison between M_g and M_t in table 4 showed similar excretion values in the two groups.

Thus dopamine alone was just as efficient as dopamine *plus* mannitol in reversing the reserpine block of theophylline diuresis.

L. Unilateral action of theophylline after dopamine-mannitol
(excretion measured for each kidney separately)

Fig. 4 shows data for the two kidneys separately in the same 7 experiments *during a continuous mannitol infusion into the wing vein* in reserpinized birds. The figure shows the excretion for the injected side and non-injected side separately, and also the ratio between injected and non-injected side after a unilateral priming with dopamine before theophylline injection into the wing vein (mean of 7 birds).

The priming injection of mannitol *(20 ml) temporarily increased* the electrolyte and urine excretion in the prediuretic period. Probably *dopamine did not influence the prediuretic excretion*. Only in one out of 7 experiments was there a greater excretion of electrolytes and urine on the dopamine injected side in the prediuretic period.

After theophylline, however, there was a *consistently higher* excretion rate on the dopamine injected side. This was most apparent for chloride *and sodium*. Further, urine was excreted somewhat more on the dopamine injected side, while potassium excretion was similar on both sides.

In the 60 minute prediuretic urine collection period the average ratio between the injected and non-injected side was close to one or slightly less for both electrolyte- and urine excretion.

After theophylline, however, the ratios were greater than one, but

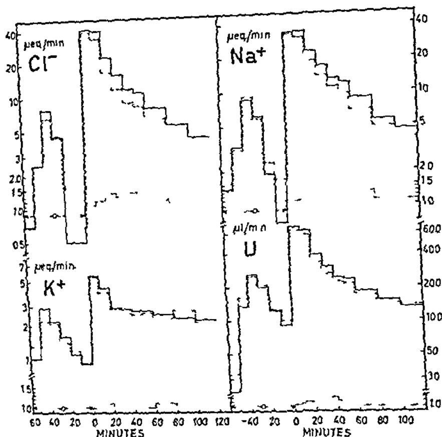


Fig 4
Reserpine pretreated birds At (—50) to (—40) minutes a priming wing vein injection of 20 ml mannitol was given from then on 0 25 ml/min was infused into the same wing vein At (—40) to (—20) minutes 5 mg dopamine was injected into one leg vein At 0—5) minutes theophylline 20 mg/kg was injected into the wing vein Electrolyte and urine excretion rate for each kidney is given separately Solid lines —— dopamine injected side dotted lines non injected side Ratios injected side to non injected side are indicated by — — — — The average ratio over the preperiod indicated is shown by ← — —0— — → (7 birds)

did not reach values as high as in the reserpinized birds given dopamine but not mannitol (*Fig 3*) Again there was a tendency for the chloride excretion to be more unilateral than *the excretion of* sodium

V TESTING OF BIRDS

All the birds primed with *dopamine into* one leg vein were tested with 0 2 mg kg phenol red *into the* same leg vein They all excreted the phenol red with a visible excess on the injected side

single injection over 5 minutes The net excretion in the hour following theophylline was calculated for both kidneys together and compared to the excretion values obtained in 14 normal birds given mannitol and theophylline only (M_c vs M_g table 4) The excretion values were very similar in the two groups except for potassium, which was in significantly higher in the dopamine experiments in reserpinized birds Thus *dopamine injection increased the diuretic effect of theophylline* in reserpine pretreated birds during mannitol infusion to the pre reserpinization level

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E Unilateral action of theophylline after dopamine mannitol

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Since species differences are evidently possible kidneys of reserpini zed and of monoamine oxidase inhibited chicken were stained with the fluorescence method for monoamines of Falck (1962) (Through the courtesy of Prosektor Bengt Falck Department of Histology Lund) Some preliminary results are now available In kidneys from the monoamine oxidase inhibited chicken adrenergic fibres were found largely perivascular and only a very few were connected to the glome rular apparatus However no adrenergic fibres have so far been ob served in direct contact with the renal tubules The kidneys from the reserpinized bird were completely amine depleted as they showed no fluorescence

Earlier attempts to reserpinize one kidney unilaterally by leg vein infusions of reserpine were unsuccessful (Nechay & Sanner 1961 b) This might have been due to lack of adrenergic innervation of the renal tubules Thus since chicken renal tubular cells are probably not adrenergically innervated the reserpine block of theophylline diuresis

VI LACK OF EFFECT OF SEROTONIN

The influence of serotonin on reserpine induced block of theophylline was studied by three injections in two reserpinized birds

In one experiment 20 mg/kg theophylline was injected into one wing vein over 5 minutes The theophylline injection was immediately followed by an infusion of 500 μg serotonin over 15 minutes given into one leg vein About two hours later an identical serotonin injection was given to the same chicken but this time 20 minutes *before* theophylline (20 mg/kg) *into the wing vein In none of these two injections* was any influence by serotonin observed on the theophylline diuresis No monoamine oxidase inhibitor was given

Another reserpinized bird was pretreated with Catron® (10 mg/kg) given into one wing vein 3 hours before serotonin was infused into the same wing vein (1 mg over 20 minutes) Thereafter 20 minutes were *allowed before 20 mg/kg theophylline* was *injected into the wing* vein Also here no influence by serotonin on the reserpine block of theophylline diuresis was observed

Discussion

DeFelice, Ferrante & Berglund (1958) reported a diuretic action of reserpine in man which was interpreted as due to a direct or indirect tubular effect of the drug.

Low doses of adrenaline and isoprenaline were antidiuretic in rats (Botting & Lockett 1961) but larger doses of adrenaline and noradrenaline in all the doses tested had a diuretic action. Botting, Farmer and Lockett (1961) also showed that the antidiuretic action of adrenaline and isoprenaline in the rat occured without any change of either glomerular filtration rate or renal plasma flow. They discussed these findings in terms of a possible sympathetic innervation of the proximal tubular cells. Lees & Lockett (1963) have provided evidence of β adrenergic receptors in the rat kidney; they showed that nethalide antagonized the actions of isoprenaline. Falck, Häggendal & Owman (1963) in studies on the frog kidney found an adrenergic supply to certain parts of the tubular system where apparently adrenaline is the transmitter.

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could be due to depletion of catecholamine stores in other tissues. The tissue most probably involved is the adrenal medulla. The chicken adrenal medulla has been shown to be amine depleted by reserpine (SCHUMANN 1958). It is therefore conceivable that the *reserpine block* of theophylline diuresis is due to an indirect renal effect by a primary depletion of the adrenal medullary stores and secondary shortage of catecholamines in the kidney.

BUNAG & WALASZEK (1962) found blood pressures of about 10—20 *mm Hg lower in chickens* injected with 2—5 mg/kg reserpine intramuscularly 16 hours previously as compared to normal animals. However, in the present experiments 1 *mg/kg reserpine given 16—19 hours* prior to the measurements did not depress the mean arterial pressure.

Theophylline diuresis was increased by mannitol in reserpinized birds. This increase might be due to an enhanced tubular urine flow. However, the reversal of the reserpine block by mannitol was only *partial*, since there still persisted a significant difference between normal and reserpinized birds both given mannitol infusion. This was strong evidence that the reserpine block was not only due to a decrease in tubular urine flow, but that also some other mechanism was important.

Mannitol infusion into the wing vein in combination with dopamine into a leg vein was found to restore the diuretic effect of theophylline *in reserpinized birds. However, reserpinized birds only primed with* dopamine also showed normal diuretic effect after theophylline. This suggested that when the catecholamine is present, mannitol infusion is no longer essential, as dopamine alone can do what mannitol *plus* dopamine does.

In these experiments a unilateral *leg vein injection of dopamine had* been given to reserpinized birds. When theophylline was given into *the wing vein 20 minutes later*, it produced a diuresis with a unilateral excess of excretion on the dopamine injected side. The excess was more marked for chloride than for sodium. Similar results were obtained in mannitol loaded birds primed with dopamine into the leg vein. This suggests a local renal effect by dopamine. The presence of catecholamine in the kidney seemed to be of importance for the diuretic effect of theophylline. Whether dopamine has to be metabolised to noradrenaline or acts by itself is an open question.

Our results provide evidence of a local renal *effect by dopamine*. The dopamine could be working at different points in the kidney.

The renal tubular cells could be directly influenced by dopamine reaching the kidney. Dopamine was recently shown to be actively

secreted by the chicken renal tubules (SANNER 1963 b) Since tubular secretion includes an active uptake and concentration mechanism it is possible that dopamine can influence theophylline diuresis in reserpinized birds by a direct action on the renal tubular cells

Dopamine could also be localized to specific tissue stores in the kidney PENNEFATHER & RAND (1960) injected dopamine into the systemic circulation of normal and reserpinized cats and analyzed the catecholamine content of the kidneys before and after dopamine In the reserpinized animals the kidney usually showed an increase in catecholamine content but in only few normal animals was an increased catecholamine concentration found in the kidney This was in keeping with the present results Normal birds primed with dopamine into the leg vein showed a similar diuretic effect on both sides when theophylline was given into the wing vein AXELROD WEIL MALHERBE & TOMCHICK (1959) infused H^3 labelled adrenaline into cats and studied its distribution in the tissues Their results indicated that circulating amine was not uniformly distributed in the tissues Those tissues where endogenous catecholamines are high also generally showed the highest degree of uptake However even 2 hours after its injection considerable amounts of unchanged adrenaline is present in most tissues They conclude that tissue binding might protect the amine from enzymatic attack and that the bound form represents a local store that can gradually be released GOODALL KIRSHNER & ROSEN (1959) obtained similar results with noradrenaline in man Injected radioactive noradrenaline rapidly disappeared from the urine but metabolic products continued to be excreted for 24 hours They concluded that the tissues pick up noradrenaline store it as a complex and gradually release it for metabolism

Also a local vascular effect of dopamine is possible The fact that the dopamine effect persists 20—80 minutes after a leg vein injection speaks against an immediate vasomotor effect However it could for instance restore tone to efferent arterioles with amine depleted vasomotor nerves Against this interpretation of the dopamine effect can be set the observation that the excess of chloride excretion was more marked than that of sodium If the block of theophylline action by reserpine had been due to a marked lowering of glomerular filtration sheer increase of filtration should have improved the diuretic effect of theophylline equally for both ions Moreover in special experiments no decrease in glomerular filtration after reserpine was found.

Summary and conclusions

[The following summary is based upon the present (I) and four earlier works by the author NECHAY & SANNER 1961 a (II) NECHAY & SANNER 1961 b (III) SANNER 1963 a (IV) and SANNER 1963 b (V)]

A Theophylline diuresis was studied by way of leg vein infusions into chickens (II) The impact of the tested substance was thus acting on one kidney with the other kidney serving as a control organ Aminophylline and theophylline were used

Leg vein infusions of aminophylline produced higher chloride excretion rates and urine flows on the infused side whereas both kidneys responded equally well to wing vein injections Theophylline as such was also tested in order to eliminate any possible artifacts due to ethylenediamine Urine flow chloride and PAH excretion were measured Chloride excretion and urine flow showed the same pattern as in the analogous aminophylline experiments This suggested a tubular point of attack by the xanthine The unilateral nature of the response does not entirely exclude a vascular point of attack but this seems unlikely At the onset of the unilateral theophylline infusion the PAH excretion showed a sudden symmetrical peak probably due to a bilateral displacement of urine from the dead space The symmetry of the PAH excretion changes indicated that they were systemic effects due to theophylline probably by influencing blood pressure or arteriolar tone Any changes in glomerular filtration rate should also if measured have occured symmetrically When chloride excretion on the two sides were compared at equal chloride excretion rates rather than at the same times then urine was found to be more concentrated on the theophylline infused side at equal excretion rates of chloride This seems to indicate that the diuresis was due to two effects operating to different extents in the two kidneys There could be a common bilateral vascular effect and a predominantly tubular effect on the infused side The biphasic response obtained during some experiments where xanthines were given into the wing vein also indicated a mixture of two mechanisms responsible for diuresis Incidentally it was

also observed that theophylline infused at 2 mg/min for 15 minutes into the wing vein of normal birds resulted in considerably smaller chloride output from both kidneys together than that caused by the same infusion directly into the renal portal circulation (III) This points to a predominantly tubular effect on the infused side

B Reserpine inhibited the diuretic effect of hydrochlorothiazide and theophylline (III)

Reserpine at a dose of 1 mg/kg did not produce any change of chloride or urine excretion when the urine was collected for 7—13 hours after the injection Comparison with prediuretic control electrolyte excretion however showed a tendency for potassium excretion to be somewhat higher in reserpine treated birds when reserpine was given many hours previously

Reserpine 1 mg/kg greatly reduced the diuretic effect of 2 mg/kg hydrochlorothiazide given as a single wing vein injection The chloride excretion was reduced to a greater extent than that of sodium and the chloride was insufficient to match the cation output Potassium excretion remained close to the controls Urine flow was markedly reduced Also continuous infusions of hydrochlorothiazide into the wing vein at 0.5 mg/min for one hour produced considerably smaller diuresis in a group of reserpinized birds

Reserpine 1 mg/kg almost completely inhibited the chloride and sodium output due to 10—20 mg/kg theophylline given systemically Potassium excretion was less reduced than the other ions while urine flow was markedly reduced The unilateral excess excretion which normally appeared after a leg vein infusion of theophylline or aminophylline (II) no longer appeared in reserpinized birds (III) Reserpine also prevented the large bilaterally symmetrical fluctuations in PAH excretion which appeared in normal birds after a leg vein infusion of theophylline

Attempts to reserpinize one kidney unilaterally by leg vein infusions of reserpine were made One bird given 0.1 mg/kg reserpine into one leg muscle 18 hours before 20 mg/kg theophylline responded with a good excess of urine and chloride output on the non reserpinized side after a wing vein injection This could however not be reproduced in 11 other experiments with different timing and doses of unilateral reserpine In another type of experiment a wing vein dose of aminophylline produced a symmetrical diuresis This was unaltered by a leg vein infusion of 0.17 mg/min of reserpine for 15 minutes (III) Thus reserpine as such did not produce any immediate change on the theophylline renal tubular response The time lag before maximal reser

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pine block was obtained points to a possible influence on the catecholamine stores Even 10—21 days after injection of 1 mg/kg reserpine *into the pectoral muscle the theophylline produced somewhat* smaller diuretic response than that of a control group (III) At that time reserpine is in all probability absent, but the catecholamine stores might be deficient Reserpine might have an indirect renal effect by a primary depletion of adrenal catecholamine stores with a secondary shortage of amines in the kidney (I).

C Dopamine priming into one leg vein produced a normalised diuretic response of systemically injected theophylline into reserpinized birds *(I) The diuresis was larger on the dopamine injected side The results* provide evidence for a local renal effect by dopamine The effect could be localized to kidney renal tubular cells by the active uptake and concentration of dopamine into these cells Dopamine is shown to be actively secreted (V) and consequently, it is also subject to uptake and concentration The dopamine could also be localized to specific tissue stores in the kidney The possibility of a local vasculator effect is also discussed but is considered to be unlikely

D Reserpine is known to deplete tissues of serotonin and catecholamines Both serotonin and catecholamines have been claimed to have renal effects, and therefore it was of interest to study their excretion mechanisms in the chicken kidney Both serotonin (IV) and dopamine (V) were found to be actively secreted by the base transport system, tolazoline inhibited the transport (IV & V) However probenecid an inhibitor of weak acid transport, was without effect on the excretion of either amine Serotonin and dopamine are probably handled by the same excretion mechanism in the chicken kidney However dopamine alone was able to restitute the reserpine block of *theophylline diuresis* (I)

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COMPARISON OF DIFFERENT LONG-ACTING CORTICOTROPIN PREPARATIONS IN THE TWO-DAY CORTICOTROPIN TEST IN MAN

BY
AIMO PEKKARINEN and URPO K. RINNE

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INTRODUCTION

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In spite of different prolonging agents the clinical potencies of the various long acting corticotropin preparations during depot corticotropin tests in man have been only little compared. In the pharmacopoea there is also no method for the bioassay of the effects of long acting corticotropin preparations. The present pharmacopoea methods only standardize the water soluble or gelatine corticotropin preparations. By means of the concentrations of 17-hydroxycorticosteroids in the plasma it has been possible to ascertain that there are differences in the adrenal cortex stimulating potencies of these preparations (Hangård *et al.* 1960, Hedner 1963). The clinical assessment of the effects of the corticotropin test has lately become still more difficult, if highly purified corticotropin preparations or synthetic corticotropin peptides have been used in the intramuscular tests. According to the commercial labelling the new highly purified corticotropin preparations should be administered intramuscularly, 1½—3 times more units than the old corticotropin preparations. Therefore it was considered necessary to compare the clinical effects of the new long acting corticotropin preparations — polyphloretin phosphate, carboxymethyl cellulose, zinc hydroxide and gelatine corticotropin in a two-day intramuscular depot corticotropin test in man by comparing the dose responses of different corticotropin preparations (20, 40, 60 and 120 I.U.) upon the urinary excretions of Porter-Silber 17-OHCS, ketogenic 17-OHCS and 17-ketosteroids. Further the effect of the new type of zinc hydroxide corticotropin

Material and Methods

The material was composed of 228 patients from the neurological clinic (113 females and 115 males), the average age was 42 years (ranges 13—67 years). 68 patients were suffering from multiple sclerosis and 39 patients from peripheral and/or cranial neuropathy. They received corticotropin treatment which was started with the two day corticotropin test. The test was carried out also on the 121 other patients (29 with the sequel of head injury, 23 with epileptic seizures 22 with neurasthenia 12 with myopathy, 10 with cerebrovascular disorders, 10 with headache, 5 with myelopathy, 5 with *anorexia nervosa*, 3 with extrapyramidal disorders, 1 with acromegaly, 1 with craniopharyngioma and 1 with myasthenia gravis) for the diagnostic evaluation of the functional capacity of the anterior pituitary adrenocortical system. Most of the patients were without treatment during the corticotropin test, but some were receiving drugs (see Tables 8—15). In none of the patient had any liver or kidney disease been observed.

For the two-day corticotropin test the daily urine samples were collected from the patients during four consecutive days. On the second and third day a certain dose of long acting corticotropin preparation was injected intramuscularly twice daily. The new type of corticotropin pre-

Table 1

Precision of methods used for the determination of 17 ketosteroids (micromodification), ketogenic 17 OHCS and Porter Silber 17 OHCS in the urine expressed as the standard error of a single determination

17 ketosteroids			Ketogenic 17 OHCS			Porter Silber 17 OHCS		
mg/liter	S.E. %	No. of analyses	mg/liter	S.E. %	No. of analyses	mg/liter	S.E. %	No. of analyses
3.4	14.0	440	9.4	18.2	188	2.4	30.0	354
9.0	8.0	263	21.0	10.2	248	6.8	16.8	326
15.5	6.1	84	34.0	6.9	212	12.0	11.6	243
21.8	5.2	40	48.0	7.1	134	22.6	10.8	404
28.5	5.7	10	61.0	7.0	87	36.8	5.4	170
37.7	5.6	19	99.0	5.5	125	52.4	3.8	70
						63.6	2.8	58
						112.2	6.5	34

parations were (see Tables 2—3). (I) Polyphloretin phosphate corticotropin (Reacthin® Leo), 20 40 and 60 I.U., (II) Carboxymethyl cellulose corticotropin (Acton prolongatum®, Cortec), 20, 40 and 60 I.U., (III) Zinc hydroxide corticotropin (Cortrophine Z®, Organon), 20, 40 and 120

was compared with that of the old type of zinc hydroxide corticotropin preparation.

Recently the method for the bioassay of prolonged corticotropin preparations in gelatine, carboxymethylcellulose, polyphloretin phosphate and zinc phosphate solutions had been developed in male guinea pigs (PEKKARINEN 1967) to determine the actual amount of added corticotropin in the prolonged corticotropin solutions. Prolonged corticotropin preparations were standardized by using the prolonged preparations of International working standards as the reference standards (Mill Hill, London) in 4- and 6-point assays on living guinea pigs after pretreatment with *pentobarbitone (Abbot 10 mg/kg) lingually*, methadone (Leiras 4 mg/kg) i.p. and chlorpromazine (May & Baker 5 mg/kg) i.p. 2 hours before blood *sampling. Corticotropin preparations were usually injected 2 hours before* blood sampling, in the case of carboxymethylcellulose 3 hours before blood sampling in the thigh *of male guinea pigs. The reference standard was* injected usually in amount of 0.033, 0.1 and 0.3 I.U./kg i.m. Blood was collected from the shoulder vein into a heparinized test tube and 17-OHCS determined fluorimetrically.

In 6 successive standardizations the mean potency of corticotropin in 15 % gelatine (Laake Oy) after dilution to 1.5 % was in 4-point assay 102.2 I.U./mg, which as commercial preparation contains 87.6 I.U./mg. In 3 successive standardizations the mean potency of Ferring corticotropin peptide (No. 40124) in 1 % PFF (Leo) was between 143.9—134.6 I.U./mg in 4-point assay and as water soluble preparation in our bioassays 147 and 136 I.U./mg in 4 series. The mean potency of 1 % carboxymethylcellulose corticotropin (Ferring *No. 129 P) was 145.3—153.6 I.U./mg* in two series of 4-point assay and as water soluble corticotropin on our earlier bioassay *140.9 I.U./mg and 143.4 I.U./mg.*

Cortrophine Z (Organon 120 I.U. per ampoule) had the mean activity *118.3 I.U./ampoule in one series and in 4 other successive series the com*bined mean potency was 112.9 I.U./ampoule in 4-point assay (ranges 107.9—117.1 I.U.).

Ciba synthetic 1—24 corticotropin peptide in gelatine had the mean activity 49.6 and 55.0 I.U./mg in 6- and 4-point assay, each in three series on guinea pigs and as a water soluble preparation without gelatine 53 and 60 and 53 and 59 I.U./mg in 6- and 4-point assay in 6 and 3 series respectively.

In addition of the knowledge of actual amount of corticotropin by standardization of the prolonged *corticotropin preparations it is impor*tant clinically to know the equivalent of their clinical effectiveness in order to compare and *unify the clinical potency* of different prolonged corticotropin preparations and their dose-relationships in man.

RESULTS

A COMPARISON OF DIFFERENT LONG ACTING CORTICOTROPIN PREPARATIONS IN THE TWO DAY CORTICOTROPIN TEST IN MAN

MEAN RESPONSES EXCRETION OF PORTER-SILBER 17 OHCS, KETOGENIC 17 OHCS AND 17 KS ABOVE THE MEAN BASAL EXCRETIONS

The adrenocortical responses and their dose relationships to polyphloretin phosphate (I, Reacthin®), carboxymethyl cellulose (II, Acton prolongatum®) zinc hydroxide (III Cortrophine-Z®) and gelatine (IV, Depo-ACTH®) corticotropins were studied in the two-day intramuscular corticotropin test (doses 20—120 I U twice daily)

According to the size of the maximum adrenocortical response to great doses of corticotropin (40—120 I U) on the second corticotropin-day, the order of the intensity of the responses was the following Polyphloretin phosphate (I) carboxymethyl cellulose (I) zinc hydroxide (III) and gelatine (IV) corticotropin preparations

I 40—120 I U (Table 2, Fig 1 2 3 and 4)

1 *Polyphloretin phosphate corticotropin (Reacthin®)*
(Table 2 Fig 1 2 3 4)

On the second corticotropin day 40 I U of polyphloretin phosphate corticotropin (I) caused the maximal mean response of excretion per 24 hrs above the mean basal excretion +49.5 mg *Porter Silber 17 OHCS*, +60 2 mg ketogenic 17 OHCS and +11 00 mg 17 KS 60 I U caused nearly similar responses

With 40 I U the responses were for Porter Silber 17 OHCS 2 7, for ketogenic 17 OHCS 2 5 and for 17 KS 2 2 times greater than those with 20 I U

2 *Carboxymethyl cellulose corticotropin (Acton prolongatum®)*
(Table 2 Fig 1 2, 3)

The mean response to 60 I U of carboxymethyl cellulose corticotropin (II) (+49 1 mg +46 6 mg and +11 9 mg for 3 different steroids res

I U of the new highly purified preparation and in addition 40 I U of the old type corticotropin for Cortrophine Z (IV) Gelatine corticotropin (Depo ACTH®, Laake Oy), 20, 60 and 120 I U

The Porter Silber 17 hydroxycorticosteroids (17 OHCS) were determined by a modification (HALME *et al* 1957) of the method of JENKINS *et al* (1955), the ketogenic 17 OHCS by our modification of the APPLEBY *et al* method (1955) and the 17 ketosteroids (17 KS) by our modification of the Callow Zimmerman method (HALME *et al* 1957) The accuracy of these methods have been published earlier (PEKKARINEN & THOMASSON 1950 PEKKARINEN, 1960, PEKKARINEN *et al* 1962) (Table 1)

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The mean response to 60 I U of carboxymethyl cellulose corticotropin (II) (+49 1 mg +46 6 mg and +11 9 mg for 3 different steroids, res

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2 *Carboxymethyl cellulose corticotropin (Acton prolongatum®)* (Table 2, Fig 1, 2, 3)

The mean response to 60 I U of carboxymethyl cellulose corticotropin (II) (+49 1 mg +46 6 mg and +11 9 mg for 3 different steroids, res-

I U of the new highly purified preparation and in addition 40 I U of the old type corticotropin for Cortrophine Z, (IV) Gelatine corticotropin (Depo ACTH®, Laake Oy), 20, 60 and 120 I U

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2 *Carboxymethyl cellulose corticotropin (Acton prolongatum®)* (Table 2 Fig 1,2 3)

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the other hand were somewhat higher than with 20 I U of polyphloretin phosphate corticotropin but the total excretion was significantly higher ($P<0\ 01$) only for the 17 KS (Table 3 4)

3 *Zinc hydroxide corticotropin (Cortrophine Z®)* (Table 2 Fig 1 2, 3, 4)

The mean response with 120 I U of the new type of zinc hydroxide corticotropin (III) (Table 2) (+52 2 mg +51 7 mg and +15 0 mg for 3 different steroids respectively) corresponded to that with 40 or 60 I U of polyphloretin phosphate corticotropin (I) and that with 60 I U of carboxy methyl cellulose (II) showing a higher mean response only for 17 KS The total excretion of those steroids with 120 I U of new type of zinc hydroxide corticotropin was not significantly ($P>0\ 05$) greater than those with 40 I U of the old type of zinc hydroxide corticotropin (III) (responses +43 7 mg +44 5 mg and +10 5 mg for 3 different steroids respectively) (Table 5 6) The response with zinc hydroxide corticotropin (III) clearly continues longer than that with polyphloretin phosphate (I) carboxy methyl cellulose (II) and gelatine corticotropin (IV) preparations on the day following the 2 corticotropin days indicating that zinc hydroxide corticotropin (III) gives a longer adrenocortical response than three other preparations (I II IV)

With 120 I U of the new type of corticotropin the responses were 1 3 for Porter Silber 17 OHCS 1 8 for ketogenic 17 OHCS and 1 7 times greater for 17 KS than with 20 I U and 2 2 1 9 and 1 3 times greater, respectively than with 40 I U

4 *Gelatine corticotropin (Depo ACTH®)* (Table 2 Fig 1 2 3 4)

The mean response with 120 I U of gelatine corticotropin (IV) (+37 7 mg +39 5 mg and +10 2 mg for 3 different steroids respectively) was smaller than with 120 I U of zinc hydroxide corticotropin (III) Only the mean excretion of Porter Silber 17 OHCS with 120 I U of gelatine corticotropin (IV) was significantly smaller on the second corticotropin-day than with 120 I U of zinc hydroxide corticotropin (III) ($P<0\ 05$) (Table 5 7) The response with 60 I U of gelatine corticotropin (IV) was only about a half of the corresponding responses of 40 or 60 I U of polyphloretin phosphate corticotropin (I) and 60 I U of carboxymethyl cellulose corticotropin (II) (Table 2)

The responses obtained with 120 I U of gelatine corticotropin were 1 7 for Porter Silber 17 OHCS 1 3 for ketogenic 17 OHCS and 1 4 times greater for 17 KS than with 60 I U and 2 1, 1 7 and 2 2 times greater, respectively than with 20 I U of gelatine corticotropin

Table 2

Mean response of 17 KS, Porter Silber 17 OHCS and Ketogenic 17 OHCS excretion above the basal excretion (mg/24 hours) in two day ACTH Test (I.U. ACTH Twice Daily i.m.) Mean

I.U.	17 KS			P.S. 17 OHCS			Ketog. 17 OHCS		
	I	II	Fo	I	II	Fo	I	II	Fo
				Polyphloretin phosphate ACTH (Reacthin®)					
60	+5.4	+12.1	+6.4	+26.4	+50.1	+10.4	+34.5	+40.4	+19.9
	(18)	(17)	(17)	(19)	(17)	(17)	(19)	(17)	(17)
40	+4.7	+11.0	+6.1	+25.6	+49.5	+17.0	+28.2	+60.2	+21.4
	(18)	(18)	(18)	(18)	(18)	(19)	(18)	(19)	(19)
20	+2.5	+5.1	+3.1	+8.8	+18.1	+1.1	+15.4	+24.1	+2.9
	(12)	(11)	(12)	(12)	(11)	(12)	(12)	(11)	(12)
				Carboxymethyl cellulose ACTH (Acton prolongatum®)					
60	+7.0	+11.9	+8.2	+27.3	+49.1	+21.0	+31.5	+40.6	+20.9
	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
20	+7.5	+8.5	+2.5	+10.4	+29.1	+7.4	+19.5	+32.4	+9.5
	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
				Zinc Hydroxide *ACTH* (Cortrophine *Z*® New Type)					
120	+4.7	+15.0	+18.7	+21.6	+52.2	+54.5	+23.0	+51.7	+57.2
	(44)	(44)	(44)	(44)	(44)	(44)	(44)	(44)	(44)
40	+5.7	+11.7	+11.4	+13.3	+23.6	+21.0	+14.8	+27.6	+22.9
	(20)	(19)	(20)	(20)	(19)	(20)	(20)	(19)	(20)
20	+5.2	+8.8	+10.0	+21.7	+40.3	+24.2	+22.7	+28.2	+32.0
	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)
				Zinc Hydroxide ACTH (Cortrophine *Z*® Oil Type)					
40	+5.9	+10.5		+20.1	+43.7		+21.1	+44.5	
	(33)	(32)		(32)	(32)		(22)	(22)	
				Gelatine ACTH (Depo ACTH®)					
120	+7.4	+10.2	+3.5	+20.8	+37.7	+4.8	+27.3	+39.5	+13.1
	(19)	(19)	(18)	(19)	(19)	(18)	(19)	(19)	(18)
60	+4.5	+7.3	+3.8	+11.8	+22.8	+2.3	+10.0	+30.3	+8.0
	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)
20	+3.0	+4.6	+1.9	+11.6	+18.1	+4.7	+15.8	+22.8	+12.2
	(13)	(13)	(13)	(13)	(13)	(13)	(13)	(13)	(13)

I and II = two ACTH days. Fo = Following day.

pectively) was nearly of the same size as that to 40 and 60 I.U. of polyphloretin phosphate corticotropin (I).

To 60 I.U. carboxymethyl cellulose corticotropin (II) the responses above the basal excretion were for all steroids only 1.4 times higher on the second corticotropin day than those obtained with 20 I.U., which on

the other hand were somewhat higher than with 20 I U of polyphloretin phosphate corticotropin but the total excretion was significantly higher (P<0 01) only for the 17 KS (Table 3, 4)

3 *Zinc hydroxide corticotropin (Cortrophine Z®)* (Table 2 Fig 1, 2 3, 4)

The mean response with 120 I U of the new type of zinc hydroxide corticotropin (III) (Table 2) (+52 2 mg +51 7 mg and +15 0 mg for 3 different steroids respectively) corresponded to that with 40 or 60 I U of polyphloretin phosphate corticotropin (I) and that with 60 I U of carboxy methyl cellulose (II) showing a higher mean response only for 17 KS The total excretion of those steroids with 120 I U of new type of zinc hydroxide corticotropin was not significantly (P>0 05) greater than those with 40 I U of the old type of zinc hydroxide corticotropin (III) (responses +43 7 mg +44 5 mg and +10 5 mg for 3 different steroids respectively) (Table 5 6) The response with zinc hydroxide corticotropin (III) clearly continues longer than that with polyphloretin phosphate (I) carboxy methyl cellulose (II) and gelatine corticotropin (IV) preparations on the day following the 2 corticotropin days indicating that zinc hydroxide corticotropin (III) gives a longer adrenocortical response than three other preparations (I II IV)

With 120 I U of the new type of corticotropin the responses were 1 3 for Porter Silber 17 OHCS 1 8 for ketogenic 17 OHCS and 1 7 times greater for 17 KS than with 20 I U and 2 2 1 9 and 1 3 times greater, respectively than with 40 I U

4 *Gelatine corticotropin (Depo ACTH®)* (Table 2 Fig 1 2 3 4)

The mean response with 120 I U of gelatine corticotropin (IV) (+37 7 mg +39 5 mg and +10 2 mg for 3 different steroids, respectively) was smaller than with 120 I U of zinc hydroxide corticotropin (III) Only the mean excretion of Porter Silber 17 OHCS with 120 I U of gelatine corticotropin (IV) was significantly smaller on the second corticotropin-day than with 120 I U of zinc hydroxide corticotropin (III) (P<0 05) (Table 5 7) The response with 60 I U of gelatine corticotropin (IV) was only about a half of the corresponding responses of 40 or 60 I U of polyphloretin phosphate corticotropin (I) and 60 I U of carboxymethyl cellulose corticotropin (II) (Table 2)

The responses obtained with 120 I U of gelatine corticotropin were 1 7 for Porter Silber 17 OHCS 1 3 for ketogenic 17 OHCS and 1 4 times greater for 17 KS than with 60 I U and 2 1 1 7 and 2 2 times greater, respectively than with 20 I U of gelatine corticotropin

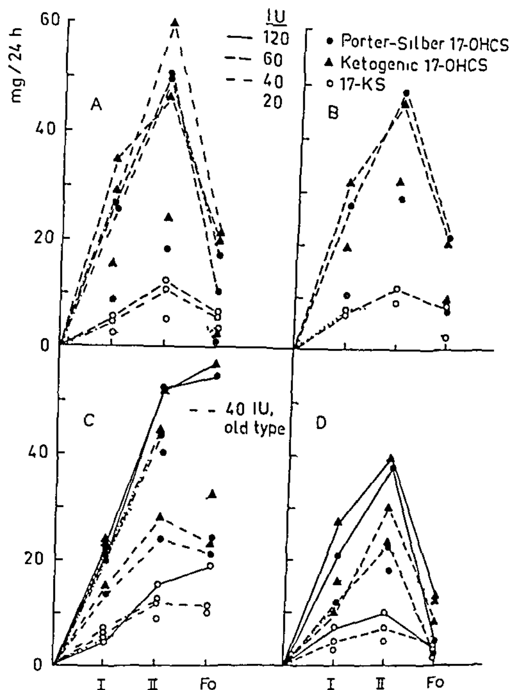


Fig 1

Response of Porter Silber 17 OHCS ketogenic 17 OHCS and 17 KS (mg 24 hours) in the two day corticotropin test Mean responses above the mean basal excretions A = polyphloretin phosphate B = carboxymethyl cellulose C – zinc hydroxide and D = gelatine ACTH Dose is injected i m twice daily I – ACTH II ACTH Fo – Following day Number of patients for each mean is in Table 2

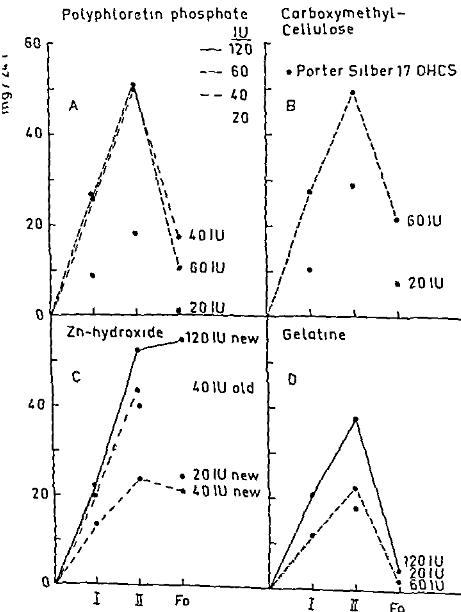


Fig 2

Response of Porter Silber 17 OHCS (mg/24 hours) in the two d…
Mean responses at…

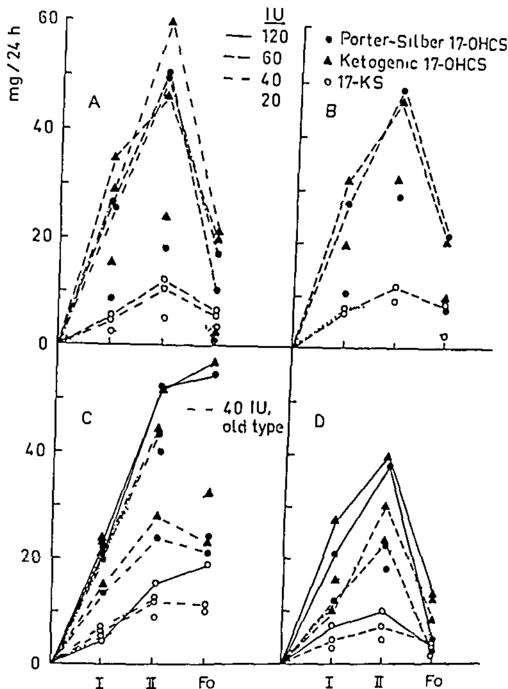


Fig 1

Response of Porter Silber 17 OHCS ketogenic 17 OHCS and 17 KS (mg/24 hours) in the two day corticotropin test Mean responses above the mean basal excretions A = polyphloretin phosphate B = carboxymethyl cellulose, C = zinc hydroxide and D = gelatine ACTH Dose is injected i m twice daily I = ACTH II = ACTH, Fo = Following day Number of patients for each mean is in Table 2

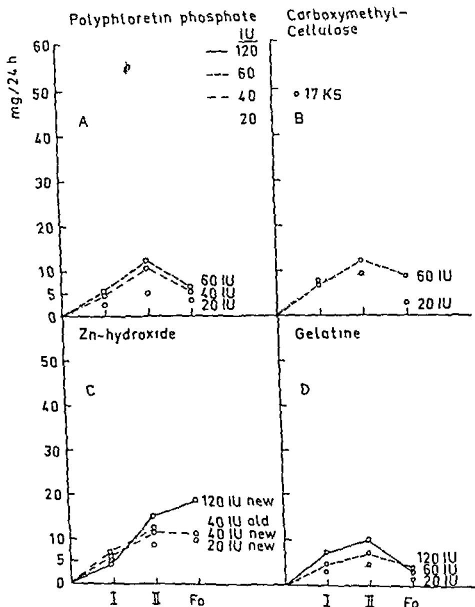


Fig 4

Response of 17 KS (mg/24 hours) in the two-day corticotropin test Mean ... above the mean basal excretions A = ... lose C – zinc hydroxide and ... I – ACTH II = ACTH Fo =

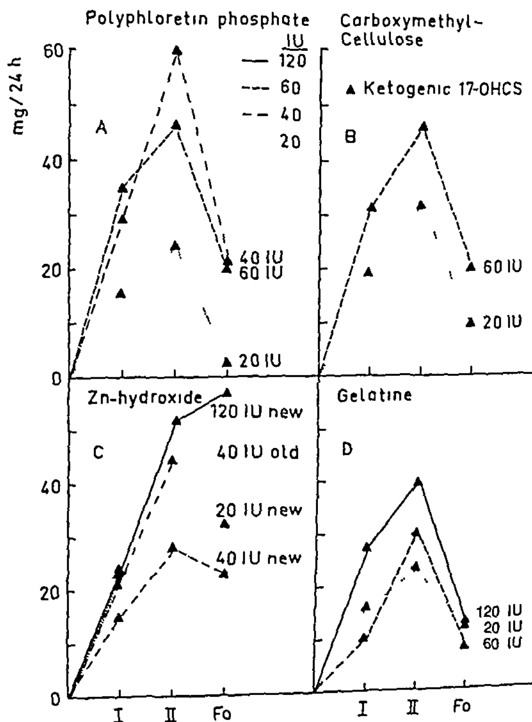


Fig 3

Response of keto ... responses above ... methyl cellulose ... daily I = ACTH II = ACTH, Fo = Following day Number of p... is in Table 2

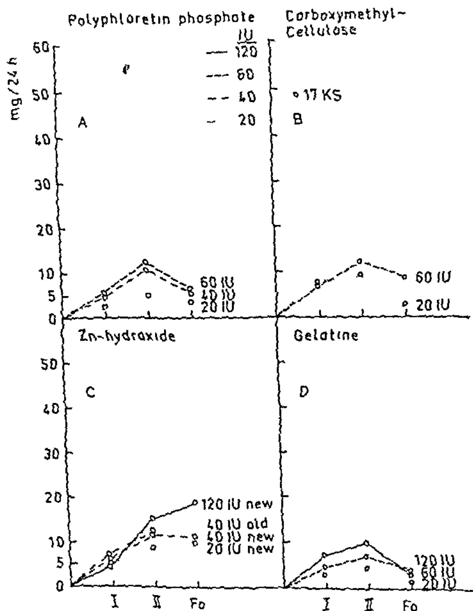


Fig 4

Response of 17 KS (mg/24 hours) in the two-day corticotropin test *Mean respons*[illegible] above the mean basal *excretions* [illegible]
lose, C = zinc hydroxide and
I = ACTH II = ACTH, Fo –

5 *All corticotropin preparations*

The mean responses of Porter Silber 17 OHCS and ketogenic 17 OHCS above the basal excretion were often of the same size and normally 3—5 times greater than the mean response of 17 KS

The mean response of Porter Silber 17 OHCS on the second corticotropin day was usually nearly twice as high as that on the first corticotropin day, while the mean response of ketogenic 17 OHCS was nearly 1 5 to 2 times higher and that of 17 KS nearly 1 5 to 3 times higher than on the first corticotropin day

II 20 I U (Table 2 Fig 1 2 3 and 4)

The adrenocortical responses caused by 20 I U with zinc hydroxide (III) and carboxymethyl cellulose (II) corticotropin were somewhat higher than with polyphloretin phosphate (I) and gelatine (IV) corticotropin on the second corticotropin day However the total excretion of Porter Silber 17 OHCS was significantly higher only between zinc hydroxide corticotropin (III) > polyphloretin phosphate corticotropin (I) (P<0 01) zinc hydroxide corticotropin (III) > gelatine corticotropin (IV) (P < 0 01) and that of 17 KS between zinc hydroxide corticotropin (III) > polyphloretin phosphate corticotropin (I) P<0 001) and carboxymethyl cellulose corticotropin (II) > polyphloretin phosphate corticotropin (I) (P<0 01) (Table 2—7)

B EFFECT OF DOSE OF DIFFERENT LONG ACTING CORTICOTROPIN PREPARATIONS ON THE TOTAL EXCRETIONS OF PORTER SILBER 17 OHCS, KETOGENIC 17 OHCS AND 17 KS

1 *Polyphloretin phosphate corticotropin (Reacthin®)* (Table 3 8 9)

a Porter Silber 17 OHCS

60 I U (19 patients) The mean basal excretion 17 6 mg increased on the first corticotropin day to 44 0 mg and on the second corticotropin day to 67 7 mg (P<0 001) (ranges 23 8—112 0 mg in 6 patients over 75 mg), but it decreased on the following day nearly to the level of the basal excretion to 28 0 mg

40 I U (18 patients) The mean basal excretion 12 7 mg increased on the second corticotropin day to 62 2 mg (P < 0 001) (ranges 23 1—97 0 mg, over 75 mg in 5 patients)

Table 8

Excretion of 17 KS Porter Silber 17 OHCS and Ketogenic 17 OHCS (mg/24 hours) in two-day ACTH Test (I U ACTH Twice Daily i m) *Polyphloretin phosphate ACTH* (Leacthin®) Mean ±SEM

U	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17 OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
60 IU 3M+6F (19)	11 2 ±0 9 (19)	16 6 ±1 4 (18)	23 3 ±2 5 (17)	17 6 ±1 8 (17)	17 6 ±2 0 (19)	44 0 ±5 8 (19)	67 7 ±5 9 (17)	28 0 ±2 4 (17)	32 4 ±3 7 (19)	66 9 ±5 2 (19)	78 8 ±12 4 (17)	52 3 ±5 0 (17)
40 IU 8M+10F (18)	9 9 ±1 1 (18)	14 6 ±1 5 (18)	20 9 ±1 1 (18)	16 0 ±1 4 (18)	12.7 ±1 4 (18)	38.3 ±5 0 (18)	62 2 ±4 8 (18)	29 7 ±3 4 (18)	27 4 ±2 7 (18)	55 6 ±6 4 (18)	87 6 ±5 3 (18)	49 9 ±4 4 (18)
20 IU 6M+6F (12)	6 3 ±1 1 (12)	8 8 ±1 3 (12)	11 4 ±1 6 (11)	9 4 ±1 7 (12)	11 0 ±1 0 (12)	19 8 ±2 2 (12)	29 1 ±3 4 (11)	12 1 ±2 8 (12)	25 1 ±5 4 (11)	40.5 ±5 7 (12)	49 2 ±7 7 (11)	28 0 ±5 4 (12)

C=Control I=ACTH, II=ACTH Fo=Following day
F=Female, M=Male

20 IU (12 patients) The mean basal excretion, 11 0 *mg* increased on the second corticotropin day to 29 1 mg ($P < 0\,001$) (maximum 44 8 mg over 25 mg in 8 patients)

b Ketogenic 17 OHCS

60 IU (19 patients) The mean basal excretion, 32 4 *mg*, increased on the first corticotropin day *to 66 9 mg* and on the second corticotropin day to 78 8 mg (maximum 170 mg, over 100 mg *in* four patients), but decreased on the following day *to 52 3 mg*

40 IU (18 patients) The mean basal excretion, 27 4 mg increased on the second corticotropin day to 87 6 mg ($P < 0\,001$) (maximum 120 0 mg in all patients over 50 mg except one, in 3 patients over 100 mg)

20 IU (12 patients) The mean basal excretion 25 1 mg, increased on the second corticotropin day to 49 2 mg ($P < 0\,05$) (maximum 102 0 mg over 50 mg in 5 patients)

c 17 Ketosteroids

60 IU (19 patients) The mean basal excretion 11 2 mg, increased on the first corticotropin-day *to 16 6 mg and* on the second corticotropin day to 23 3 mg ($P < 0\,001$) (maximum to 46 5 mg, above 25 mg in 7 patients) but decreased on the following day to 17 6 *mg*

5 *All corticotropin preparations*

The mean responses of Porter Silber 17 OHCS and ketogenic 17 OHCS above the basal excretion were often of the same size and normally 3—5 times greater than the *mean response of* 17 KS

The mean response of Porter Silber 17 OHCS on the second corticotropin day was usually nearly twice as high as that on the first corticotropin day while the mean response of ketogenic 17 OHCS was nearly 1 5 to 2 times higher and that of 17 KS nearly 1 5 to 3 times higher than on the first corticotropin day

II 20 I U (Table 2 Fig 1 2 3 and 4)

The adrenocortical responses caused by *20 I U with zinc hydroxide* (III) and carboxymethyl cellulose (II) corticotropin were somewhat higher than with polyphloretin phosphate (I) and gelatine (IV) corticotropin on the second corticotropin day However the total excretion of Porter Silber 17 OHCS was significantly higher only between zinc hydroxide corticotropin (III) > polyphloretin phosphate corticotropin (I) (P<0 01) zinc hydroxide corticotropin (III) > gelatine corticotropin *(IV) (P < 0 01) and that of 17 KS between zinc hydroxide corticotropin* (III) > polyphloretin phosphate corticotropin (I) P<0 001) and carboxymethyl cellulose corticotropin (II) > polyphloretin phosphate corticotropin (I) (P<0 01) (Table 2—7)

B EFFECT OF DOSE OF DIFFERENT LONG ACTING CORTICOTROPIN PREPARATIONS ON THE TOTAL EXCRETIONS OF PORTER SILBER 17 OHCS KETOGENIC 17 OHCS AND 17 KS

1 *Polyphloretin phosphate corticotropin (Reacthin®)* (Table 3 8 9)

a Porter Silber 17 OHCS

60 I U (19 patients) The mean basal excretion 17 6 mg increased on the first corticotropin day to 44 0 mg and on the second corticotropin day to 67 7 mg (P<0 001) (ranges 23 8—112 0 mg in 6 patients over 75 mg) but it decreased on the following day nearly to the level of the basal excretion to 28 0 mg

10 I U (18 patients) The mean basal excretion 12 7 mg increased *on the second corticotropin day* to 62 2 mg (P < 0 001) (ranges 23 1 — 97 0 mg over 75 mg in 5 patients)

Table 4

Excretion of 17 KS, Porter Silber 17 OHCS and Ketogenic 17-OHCS (mg/24 hours) in two-day ACTH Test (I U ACTH Twice Daily i.m.) *Carboxymethyl cellulose ACTH* (Acton prolongatum®) Mean ± SEM

U	17 KS mg/24 hrs				Porter Silber 17-OHCS mg/24 hrs				Ketogenic 17-OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
0 I U M + 13 F = (15)	6 7 ± 0 7 (15)	13 7 ± 1 7 (15)	18 6 ± 2.3 (15)	14.9 ± 1.5 (15)	8.3 ± 1.3 (15)	25 6 ± 3.2 (15)	57 4 ± 6.3 (15)	29.3 ± 4.7 (15)	20.5 ± 2.1 (15)	59 0 ± 4.5 (15)	67 1 ± 8.5 (15)	41 4 ± 4 7 (15)
20 I U 9 M + 1 F = (10)	12.5 ± 1.2 (10)	20 0 ± 2 6 (10)	21 0 ± 1.4 (10)	15 0 ± 1 9 (10)	11 6 ± 1.5 (10)	22.0 ± 2.7 (10)	40 7 ± 8 0 (10)	19 0 ± 3.3 (10)	39 7 ± 5.2 (10)	59.2 ± 9 5 (10)	72.1 ± 10 6 (10)	49.2 ± 4 6 (10)

C = Control, I = ACTH, II = ACTH, Fo = Following day
F = Female, M = Male

20 I U (10 patients) The mean basal excretion increased from 39 7 mg on the second corticotropin day to 72 1 mg (P<0 05) (maximum 125 0 mg, above 75 mg in 4 patients and above 50 mg in 8 patients

e 17 Ketosteroids

60 I U (15 patients) The mean basal excretion of 6 7 mg *increased* on *the first* corticotropin-day to 13 7 mg and on the second corticotropin day to 18 6 mg (P<0 001) (maximum 30 5 *mg, above 25* mg in *5* patients), but decreased on the following *day to 14 9 mg*

20 I U (10 patients) The excretion increased to 21 *mg on the second* corticotropin-day (P<0 001) (ranges 15 5—31 5 mg, above 20 mg in 7 patients)

Comparison

60 I U of carboxymethyl *cellulose corticotropin increases* the total excretions of steroids *on the second* corticotropin-day to a level which is somewhat lower than those with *60 I U* polyphloretin phosphate corticotropin It is partially influenced also by the variation of the mean basal excretions because the basal *excretions are higher* in the group of 60 I U of polyphloretin phosphate *corticotropin than* in the group *of 60 I U* of carboxymethyl cellulose corticotropin *20 I U* of carboxymethyl cellulose corticotropin increases the excretions of ketogenic 17 OHCS and 17 KS to

40 I U (18 patients) The mean basal excretion, 9 9 mg, increased on the second corticotropin day to 20 9 mg ($P<0\ 001$) (maximum 30 mg in all patients above 15 0 mg, except one, in 4 patients above 25 mg)

20 I U (12 patients) The mean basal excretion of 6 3 mg increased on the second corticotropin day to 11 4 mg ($P<0\ 05$) (maximum 17 5 mg, in 4 patients above 15 mg)

Comparison

The excretion of Porter Silber 17 OHCS is of the same size with 40 and 60 I U polyphloretin phosphate corticotropin on the second cortico tropin day and the excretion of ketogenic 17 OHCS even somewhat higher with 40 I U than with 60 I U On the other hand, these excretions and the excretion of 17 KS with 20 I U are approximately one half with 40 I U of corticotropin 40 I U of corticotropin can already present a maximal adre nocortical reserve of steroid excretions In 4 or 6 point assays of long acting corticotropin preparations the doses must increase in the logarithm of 3 in order to be able to differentiate clearly between the adrenocortical responses to different corticotropin doses as e g for water soluble corticotropins in the bioassays based upon the plasma free 17 OHCS determination in guinea pigs (PEKKARINEN 1964, 1965)

2 *Carboxymethyl cellulose corticotropin (Acton prolongatum®)* (Table 4, 10)

a Porter Silber 17 OHCS

60 I U (15 patients) The mean basal excretion, 8 3 mg, increased on the first corticotropin day to 35 6 mg, and on the second corticotropin day to 57 4 mg ($P<0\ 001$) (maximum excretion 106 1 mg above 75 mg in 2 patients) and decreased on the following day to 29 3 mg

20 I U (10 patients) The mean basal excretion increased from 11 6 mg on the second corticotropin day to 40 7 mg ($P<0\ 001$) (maximum 66 6 mg, above 50 mg in 3 patients and above 25 mg in 9 patients)

b Ketogenic 17 OHCS

60 I U (15 patients) The mean basal excretion 20 5 mg increased on the first corticotropin day to 52 0 and on the second corticotropin day to 67 1 mg ($P<0\ 001$) (maximum 142 0 mg, above 100 mg in 2 patients) but decreased on the following day to 41 4 mg

Table 4

Excretion of 17 KS, Porter Silber 17 OHCS and Ketogenic 17-OHCS (mg/24 hours) in two day ACTH Test (I.U ACTH Twice Daily i.m.) *Carboxymethyl cellulose ACTH* (Acton prolongatum®) Mean±SEM

U	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17-OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
0 I U M+13 F (15)	6.7 ±0 7 (15)	13 7 ±1 7 (15)	18 6 ±2.3 (15)	14 9 ±1 5 (15)	8.3 ±1 3 (15)	35 6 ±3 2 (15)	57 4 ±6.3 (15)	29 3 ±4 7 (15)	20 5 ±2 1 (15)	52 0 ±4 5 (15)	67 1 ±8 5 (15)	41 4 ±4 7 (15)
0 I U M+1 F (10)	12 5 ±1 2 (10)	20 0 ±2 6 (10)	21 0 ±1 4 (10)	15 0 ±1 9 (10)	11 6 ±1.5 (10)	22 0 ±2 7 (10)	40 7 ±5 0 (10)	19 0 ±3 3 (10)	39 7 ±5.2 (10)	59.2 ±9 5 (10)	72 1 ±10 6 (10)	49 2 ±4 6 (10)

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

20 I U (10 patients) The mean basal excretion increased *from* 39 7 mg on the second corticotropin day to 72 1 mg ($P<0\ 05$) (maximum 125 0 mg, above 75 mg in 4 patients and above 50 mg in 8 patients

c 17 Ketosteroids

60 I U (15 patients) The mean basal excretion of 6 7 mg *increased* on the first corticotropin-day to 13 7 mg and on the second corticotropin day *to 18 6 mg* ($P<0\ 001$) (maximum 30 5 mg, abo*ve 25 mg in 5 patients*), but decreased on the following day to 14 9 mg

20 I U (10 patients) The excretion increased *to 21 mg on the second* corticotropin day ($P<0\ 001$) (*ranges* 15 5—31 5 *mg*, abo*ve* 20 mg in 7 patients)

Comparison

60 I U of carboxymethyl cellulose *corticotropin* increases the total excretions of steroids on the *second corticotropin-day to a level* which is somewhat lower than those with *60 I U* polyphloretin phosphate corticotropin It is partially *influenced also by the* variation of the mean basal excretions because the *basal excretions are higher in the group* of 60 I U *of* polyphloretin phosphate corticotropin *than in* the group of 60 I U of carboxymethyl cellulose corticotropin 20 *I U of* carboxymethyl cellulose corticotropin increases the excretions of ketogenic 17 OHCS and 17 KS to

the same level as 60 I U on the second corticotropin day, while the excretion of Porter Silber 17 OHCS with 20 I U is smaller than with 60 I U However the basal excretions of ketogenic 17 OHCS and 17 KS are higher with 20 I U than with 60 I U In the clinical evaluation of the variable steroid excretion on the second corticotropin day the differences between 20 and 60 I U corticotropin are therefore very small although the dose ratio is 1 3 or greater However there are clearer difference when the responses above basal excretions have been used as indicator of the size of the adreno cortical reserve (Table 2)

3 *Zinc hydroxide corticotropin (Cortrophine Z®)*
(Table 5 6 11 12 13)

a Porter Silber 17 OHCS

120 I U new type of corticotropin (44 patients) The mean basal excretion 9 7 mg increased on the first corticotropin day to 31 3 mg and on the second corticotropin day to 61 9 mg (P < 0 001) (maximum to 117 8 mg above 100 mg in 5 patients and above 75 mg in 13 patients) and was further increased on the following day 64 2 mg (P < 0 001)

40 I U old type of corticotropin (22 patients) (Table 6) The mean basal excretion was 8 0—7 6 mg for two control days and increased on the first corticotropin day to 27 9 mg and on the second corticotropin day to 51 5 mg (P < 0 001) (maximum 148 9 mg above 100 mg in 3 patients above 50 mg in addition in 11 other patients) (Table 6)

40 I U new type of corticotropin (20 patients) The mean basal excretion of 10 2 mg increased on the second corticotropin day to 33 8 mg (P < 0 001) (maximum 58 8 mg above 50 mg in 3 patients) and remained on the following day on the same level 31 2 mg

20 I U new type of corticotropin (16 patients) The mean basal excretion increased from 13 6 mg on the second corticotropin day to 53 9 mg (P < 0 001) (maximum 90 8 mg above 75 mg in three and above 50 mg in five other patients) and was on the following day 37 8 mg

b Ketogenic 17 OHCS

(1) *120 I U new type of corticotropin* (44 patients) The mean basal excretion 30 2 mg increased on the first corticotropin day to 53 2 mg on the second corticotropin day to 81 9 mg (P < 0 001) (maximum 160 0 mg above 100 mg in 12 patients and above 75 mg in addition in 12 other patients) and increased further on the following day to 87 4 mg (P < 0 001)

Table 5

Excretion of 17 KS, Porter Silber 17 OHCS and Ketogenic 17 OHCS (mg/24 hours) in two-day ACTH Test (IU ACTH Twice Daily i.m.) *Zinc-Hydroxide ACTH, (Cortrophine Z®) New Type* Mean ±SEM

	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17 OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	F
Type IU												
	9 6 ±0 3 (1*)	15 9 ±2 6 (17)	28 6 ±4 3 (17)	32 5 ±5 3 (17)	11 1 ±2 6 +3 8...	30 8 +3 8 (17)	64 5 ±7 3 (17)	63 2 ±8 7 (17)	35 5 ±3 8 (17)	62 2 ±3.5 (17)	90 9 ±8 0 (17)	8[illegible] ±[illegible] (1[illegible]
)	7 9 ±0 8 (27)	11 9 ±1 3 (27)	20 9 ±1 8 (27)	24 1 ±1 8 (27)	8 8 ±1 2 (27)	31 7 ±1 0 (27)	60 2 ±4 3 (27)	64 8 ±4 8 (27)	26 5 ±2 0 (27)	47 6 ±3 1 (27)	76 2 ±5 4 (27)	8 ±[illegible] ([illegible]
F)	8 6 ±0 6 (44)	13 3 ±1.3 (44)	23 6 ±2 0 (44)	27 3 ±2 4 (44)	9 7 ±1 2 (44)	31.3 ±2 4 (44)	61 9 ±3 8 (44)	64.2 ±4 4 (44)	30.2 ±2 0 (44)	53.2 ±2.6 (44)	81 9 ±9 5 (44)	8 ± ([illegible]
Type IU												
)	7 9 ±1 9 (10)	13 4 ±1 6 (10)	18 7 ±1 5 (10)	21 2 ±2 4 (10)	9 8 ±1 4 (10)	20 3 ±2 5 (10)	29 3 ±5 2 (10)	31 9 ±4 5 (10)	33.3 ±5 9 (10)	47 9 ±7 1 (10)	61 8 ±7 9 (10)	[illegible] ± (
)	9 2 ±1 5 (10)	15 0 ±3 6 (10)	29 9 ±7 3 (9)	18 5 ±5 1 (10)	10 6 ±1 8 (10)	26 6 ±2 7 (10)	38 9 ±4 9 (9)	30 5 ±4 6 (10)	21 5 ±3 5 (10)	36 5 ±4 8 (10)	48 1 ±3 6 (9)	4 ± (
+ F)	8 5 ±1 0 (20)	14 2 +1 9 (20)	20 2 ±3 5 (19)	19 9 +2 7 (20)	10 2 ±1 1 (20)	23 5 ±1 9 (20)	33 8 ±3 7 (19)	31 2 ±3.2 (20)	27 4 ±3 6 (20)	42 2 ±4 4 (20)	55 0 ±4 7 (19)	[illegible] ± (
w Type 0 1U												
M + 5 F (16)	10 0 ±0 9 (16)	15.2 ±1 3 (16)	18 8 ±2 0 (16)	20 0 ±1 9 (16)	13 6 ±1 3 (16)	35.3 ±3 4 (16)	53 9 ±5 0 (16)	37 8 ±3 7 (16)	34 4 ±2 5 (16)	57 1 ±3 4 (16)	62 6 ±6 5 (16)	± (

C Control I = ACTH, II = ACTH, Fo = Following day
F = Female M – Male

40 I U old type of corticotropin (22 patients) (Table 6, 13) On the two control days the excretions, 29 7 mg and 26 3 mg, increased on the first corticotropin day to 49 1 mg and on the second corticotropin day to 72 5 mg ($P<0\ 001$) (maximum 147 0 mg, above 100 mg in 4 patients and above 75 mg in 5 patients) (Table 5)

the same level as 60 I U on the second corticotropin day, while the excretion of Porter Silber 17 OHCS with 20 I U is smaller than with 60 I U However the basal excretions of ketogenic 17 OHCS and 17 KS are higher with 20 I U than with 60 I U In the clinical evaluation of the variable steroid excretion on the second corticotropin day the differences between 20 and 60 I U corticotropin are therefore very small although the dose ratio is 1 3 or greater However there are clearer difference when the responses above basal excretions have been used as indicator of the size of the adrenocortical reserve (Table 2)

3 *Zinc hydroxide corticotropin (Cortrophine Z®)* *(Table 5 6 11, 12, 13)*

a Porter Silber 17 OHCS

120 I U, new type of corticotropin (44 patients) The mean basal excretion 9 7 mg increased on the first corticotropin day to 31 3 mg and on the second corticotropin day to 61 9 mg ($P < 0.001$) (maximum to 117 8 mg above 100 mg in 5 patients and above 75 mg in 13 patients) and was further increased on the following day 64 2 mg ($P < 0.001$)

40 I U, old type of corticotropin (22 patients) (Table 6) The mean basal excretion was 8 0—7 6 mg for two control days and increased on the first corticotropin day to 27 9 mg and on the second corticotropin day to 51 5 mg ($P < 0.001$) (maximum 148 9 mg above *100* mg in *3 patients* above 50 mg in addition in 11 other patients) (Table 6)

40 I U new type of corticotropin (20 patients) The mean basal excretion of 10 2 mg increased on the second corticotropin day to 33 8 mg ($P < 0.001$) (maximum 58 8 mg above 50 mg in 3 patients) and remained on the following day on the same level *31 2 mg*

20 I U new type of corticotropin (16 patients) The mean basal excretion increased from 13 6 mg on the second corticotropin day to 53 9 mg ($P < 0.001$) (maximum 90 8 mg above 75 mg in three and above 50 mg in five other patients) and was on the following day 37 8 mg

b Ketogenic 17 OHCS

(1) *120 I U new type of corticotropin* (44 *patients*) The mean basal excretion 30 2 mg increased on the first corticotropin day to 53 2 mg on the second corticotropin day to 81 9 mg ($P < 0.001$) (maximum 165 0 mg above 100 mg in 12 patients and above 75 mg in addition in *12 other* patients) and increased further on the following day to 87 4 mg ($P < 0.001$)

Table 5

Excretion of 17 KS, Porter Silber 17 OHCS and Ketogenic 17 OHCS (mg/24 hours) in two day ACTH Test (I U ACTH Twice Daily i m) *Zinc Hydroxide ACTH*, (Cortrophine Z®) New Type Mean ±SEM

I U	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17 OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
New Type 120 I U M (17)	9.6 ±0.3 (17)	15.9 ±2.6 (17)	28.6 ±4.3 (17)	32.5 ±5.3 (17)	11.1 ±2.6 (17)	30.8 ±3.8 (17)	64.5 ±7.3 (17)	63.2 ±8.7 (17)	35.5 ±3.8 (17)	62.2 ±3.5 (17)	90.9 ±8.0 (17)	87.3 ±8.6 (17)
F (27)	7.9 ±0.8 (27)	11.9 ±1.3 (27)	20.9 ±1.8 (27)	24.1 ±1.8 (27)	8.8 ±1.2 (27)	31.7 ±1.0 (27)	60.2 ±4.3 (27)	64.8 ±4.8 (27)	26.5 ±2.0 (27)	47.6 ±3.1 (27)	76.2 ±5.4 (27)	87.4 ±8.2 (27)
M+F (44)	8.6 ±0.6 (44)	13.3 ±1.3 (44)	23.6 ±2.0 (44)	27.3 ±2.4 (44)	9.7 ±1.2 (44)	31.3 ±2.4 (44)	61.0 ±3.8 (44)	64.2 ±4.4 (44)	30.2 ±2.0 (44)	53.2 ±2.6 (44)	81.9 ±9.5 (44)	87.4 ±1.6 (44)
New Type 40 I U M (10)	7.9 ±1.9 (10)	13.4 ±1.6 (10)	18.7 ±1.5 (10)	21.2 ±2.4 (10)	9.8 ±1.4 (10)	20.3 ±2.5 (10)	29.3 ±5.2 (10)	31.9 ±4.5 (10)	33.3 ±5.9 (10)	47.9 ±7.1 (10)	61.3 ±7.9 (10)	59.6 ±6.0 (10)
F (10)	9.2 ±1.5 (10)	15.0 ±3.6 (10)	20.9 ±7.3 (9)	18.5 ±5.1 (10)	10.6 ±1.8 (10)	26.6 ±2.7 (10)	39.9 ±4.9 (9)	30.5 ±4.6 (10)	21.5 ±3.5 (10)	36.5 ±4.8 (10)	49.1 ±3.6 (9)	41.0 ±5.6 (10)
M+F (20)	8.5 ±1.0 (20)	14.2 ±1.9 (20)	20.2 ±3.5 (19)	19.9 ±2.7 (20)	10.2 ±1.1 (20)	27.5 ±1.9 (20)	33.8 ±3.7 (19)	31.2 ±3.2 (20)	27.4 ±3.6 (20)	42.2 ±4.4 (20)	55.0 ±4.7 (19)	50.3 ±4.5 (20)
New Type 29 I U 11 M+5 F (16)	10.0 ±0.9 (16)	15.2 ±1.3 (16)	18.8 ±2.0 (16)	20.0 ±1.9 (16)	13.6 ±1.3 (16)	35.3 ±3.4 (16)	53.9 ±5.0 (16)	37.8 ±3.7 (16)	34.4 ±2.5 (16)	57.1 ±3.4 (16)	62.6 ±6.5 (16)	66.4 ±6.1 (16)

C = Control I = ACTH II = ACTH, Fo Following day
F = Female M = Male

10 I U old type of corticotropin (22 patients) (Table 6, 13) On the two control days the excretions, 29.7 mg and 26.3 mg, increased on the first corticotropin day to 49.1 mg and on the second corticotropin day to 72.5 mg ($P < 0.001$) (maximum 147.0 mg, above 100 mg in 4 patients and above 75 mg in 5 patients) (Table 5)

Table 6

Excretion of 17 KS, Porter Silber 17 OHCS and Ketogenic 17 OHCS *(mg/24 hours)* in two day ACTH Test (I U ACTH Twice Daily i m) *Zinc-Hydroxide ACTH (Cortrophine Z®), New and Old Type* In the series of old type of Cortrophine Z® two control days are first and then two ACTH days Mean ±SEM

I U	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17 OHCS mg/24 hrs			
	C	C	I	II	C	C	I	II	C	C	I	II
Old Type 40 I U												
M (13)	6 7 ±1 1 (13)	7 2 ±1 4 (13)	11 1 ±1 9 (13)	14 7 ±2 8 (13)	9 1 ±1 7 (13)	6 3 ±1 4 (12)	29 0 ±3 8 (13)	53 3 ±8 1 (13)	27 3 ±4 4 (9)	24 9 ±5 0 (8)	45 2 ±7 8 (9)	70 7 ±12 1 (9)
F (20)	9 5 ±1 5 (20)	9 2 +1 5 (19)	16 6 ±2 4 (20)	22 1 ±2 9 (19)	7 1 ±1 1 (18)	8 5 ±1 4 (18)	27 1 ±3 3 (19)	50 3 ±7 6 (19)	31 5 ±2 4 (12)	27 2 ±4 0 (13)	51 8 ±5 8 (13)	73 9 ±5 7 (13)
M+F (33)	8 4 ±1 1 (33)	8 7 +1 1 (32)	14.5 ±1 8 (33)	19 1 ±2 3 (32)	8 0 ±0 9 (31)	7 6 ±1 0 (30)	27 9 ±2 5 (32)	51 5 ±5 5 (32)	29 7 ±2 3 (21)	26 3 ±2 8 (21)	49 1 ±4 6 (22)	72 5 ±5 8 (22)

C=2 Control days, I=ACTH, II=ACTH

	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
New Type 120 I U												
M (17)	9 6 ±0 3 (17)	15 9 ±2 6 (17)	28 6 ±4 3 (17)	32 5 ±5 3 (17)	11 1 ±2 6 (17)	30 8 ±3 8 (17)	64 5 ±7 3 (17)	63 2 ±8 7 (17)	35 5 ±3 8 (17)	62 2 ±3 5 (17)	90 9 ±9 0 (17)	87 3 ±8 6 (17)
F (27)	7 9 ±0 8 (27)	11 9 ±1.3 (27)	20 9 ±1 8 (27)	24.1 ±1 8 (27)	8 8 ±1 2 (27)	31 7 ±1 0 (27)	60 2 ±4 3 (27)	61 8 ±4 8 (27)	26 5 ±2 0 (27)	47 6 ±3 1 (27)	76 2 ±5 4 (27)	87 4 ±8.2 (27)
M+F (44)	8 6 ±0 6 (44)	13 3 ±1 3 (44)	23 6 ±2 0 (44)	27 3 ±2 4 (44)	9 7 ±1 2 (44)	31 3 ±2 4 (44)	61 9 ±3 8 (44)	61 2 ±4 4 (44)	30 2 ±2 0 (44)	53 2 ±2 6 (44	81 9 ±9 5 (44)	87 4 ±1 6 (44)

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

(2) *10 I U, new type of corticotropin* (20 patients) The mean basal excretion increased from 27 4 mg on the second corticotropin day to 55 0 mg (P < 0 001) (maximum 110 0 mg, above 75 mg in two and above 50 mg in 11 other patients) and was nearly on the same level on the following day, 50 3 mg

(3) *20 I U, new type of corticotropin* (16 patients) The mean basal excretion, 34 4 mg, increased on the second corticotropin-day to 62 6 mg ($P < 0\,001$) (maximum 118 0 mg and above 75 mg in 4 patients) and increased further on the following day to 66 4 mg ($P < 0\,001$)

c 17 Ketosteroids

(1) *120 I U, new type of corticotropin* (44 patients) The mean basal excretion, 8 6 mg, increased on the first corticotropin-day to 13 3 mg, on the second corticotropin-day to 23 6 mg ($P < 0\,001$) (maximum 74 5 mg, above 20 mg in 22 patients) and on the following day further to 27 3 mg ($P < 0\,001$)

40 I U, old type of corticotropin (33 patients) The mean excretions on the control days were 8 4 mg and 8 7 mg and increased on the first corticotropin day to 14 5 mg and on the second corticotropin day to 19 1 mg ($P < 0\,001$) (maximum 52 3 mg, above 20 mg in 13 patients) (Table 6)

(2) *10 I U, new type of corticotropin* (20 patients) The mean basal excretion of 8 5 mg increased on the second corticotropin-day to 20 2 mg ($P < 0\,001$) (maximum 78 5 mg, above 20 mg in 8 patients) and was still on the same level on the following day

(3) *20 I U, new type of corticotropin* (16 patients) The mean basal excretion increased from 10 0 mg on the second corticotropin-day to 18 8 mg ($P < 0\,001$) (maximum 32 0 mg, above 20 mg in 5 patients) and further on the following day to 20 0 mg ($P < 0\,001$)

Comparison

New type of corticotropin (Cortrophine Z®)

While 120 I U of corticotropin increases the excretion of Porter Silber and ketogenic 17 OHCS on the second corticotropin day to 61 9 and 81 9 mg, 40 I U increases them respectively only to 33 8 and 55 0 mg On the other hand 20 I U corticotropin causes a higher mean excretion of Porter-Silber 17 OHCS than 40 I U and the excretions of ketogenic 17 OHCS and 17 KS with 20 I U (respectively 62 6 mg and 18 8 mg) are nearly of the same size as with 40 I U (respectively 55 0 mg and 20 2 mg) Therefore responses with different doses of corticotropins show a relatively wide biological variation The small differences in the doses of corticotropin are often not able to differentiate the corresponding adrenocortical responses

The new and old type of corticotropin (Cortrophine Z®)

40 I U of old type of zinc hydroxide corticotropin (the first two control days and then two corticotropin days) increases the excretion of Porter-

Silber 17 OHCS to 51.5 mg and that of ketogenic 17 OHCS to 72.5 mg on the second corticotropin day, and the new type of zinc hydroxide corticotropin to 120 I U, to 61.9 mg and 81.9 mg, respectively. These excretions do not differ significantly (P<0.05). Therefore the adrenocortical responses with 120 I U of new corticotropin and 40 I U of old corticotropin are nearly of the same size, as expected. The old and new corticotropin used in recommended doses (ratio 1:3) have nearly equal clinical potency with a tendency to somewhat higher mean response with 120 I U of new corticotropin than with 40 I U old corticotropin. The difference between these responses is not great when we take into account the individual biological variations of the adrenocortical reserves.

4 *Gelatine corticotropin (Depo ACTH®)* (Table 7, 14, 15)

a Porter Silber 17 OHCS

120 I U (19 patients). The mean basal excretion, 14.6 mg, increased on the first corticotropin day to 35.4 mg, on the second corticotropin day to 52.3 mg (P < 0.001) (maximum to 92.0 mg, above 50 mg in 10 patients) and decreased on the following day to 19.4 mg.

Table 7

Excretion of 17 KS, Porter Silber 17 OHCS and Ketogenic 17 OHCS (mg/24 hours) in two day ACTH Test (I U ACTH Twice Daily i m) *Gelatine ACTH (Depo ACTH®)* Mean ±SEM

I U	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17 OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
120 I U 13 M + 6 F = (19)	9.0 ±0.9 (18)	16.4 ±1.4 (19)	19.2 ±1.6 (19)	12.5 ±1.1 (18)	14.6 ±1.0 (18)	35.4 ±2.4 (19)	52.3 ±3.9 (19)	19.4 ±2.4 (18)	[illegible] ±2.0 (18)	55.0 ±3.5 (19)	67.2 ±6.1 (19)	40.8 ±4.4 (18)
60 I U 6 M + 3 F = (9)	10.8 ±1.3 (9)	15.3 ±1.9 (9)	18.1 ±1.6 (9)	14.6 ±1.6 (9)	10.9 ±1.6 (9)	22.7 ±[illegible].5 (9)	33.7 ±2.4 (9)	13.2 ±2.5 (9)	30.7 ±1.6 (9)	40.7 ±6.5 (9)	61.0 ±6.9 (9)	38.7 ±4.6 (9)
20 I U 9 M + 4 F = (13)	9.3 ±1.1 (13)	12.3 ±1.7 (13)	13.9 ±2.2 (13)	11.2 ±2.1 (13)	11.2 ±1.3 (13)	22.8 ±2.4 (13)	20.3 ±3.7 (13)	15.9 ±3.5 (13)	30.1 ±3.4 (13)	[illegible] ±5.0 (13)	52.9 ±[illegible].3 (13)	4[illegible].3 ±5.4 (13)

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

60 I U (9 patients) The mean basal excretion increased from 10 9 mg to 33 7 mg ($P < 0\,001$) on the second corticotropin day (maximum 51 6 mg, above 25 mg in 10 patients)

20 I U (13 patients) The mean basal excretion, 11 2 mg, increased on the second corticotropin day to 29 3 mg ($P < 0\,001$) (maximum 51 6 mg, above 25 mg in 10 patients)

b Ketogenic 17-OHCS

120 I U (19 patients) The mean basal excretion 27 mg, increased on the first corticotropin day to 55 0 mg on the second corticotropin day to 67 2 mg ($P < 0\,001$) (maximum 104 1 mg, above 75 mg in 8 patients) and decreased on the following day to 40 8 mg

60 I U (9 patients) The mean basal excretion increased from 30 7 mg to 61 9 mg ($P < 0\,01$) on the second corticotropin day (maximum 87 0 mg above 75 mg in 3 patients)

20 I U (13 patients) The mean basal excretion of 30 1 mg increased on the second corticotropin day to 52 9 mg ($P < 0\,01$) (*maximum* 115 0 mg, above 50 mg in 6 patients)

c 17 Ketosteroids

120 I U (19 patients) The mean basal excretion 9 0 mg increased on the first corticotropin day to 16 4 mg, on the second corticotropin day to 19 2 mg ($P < 0\,001$) (maximum was 30 0 mg above 20 mg in 8 patients) and decreased on the following day to 12 5 mg

60 I U (13 patients) The mean basal excretion increased from 10 8 mg on the second corticotropin day to 18 1 mg ($P < 0\,01$) (*maximum* 26 5 mg above 20 mg in 2 patients)

20 I U (13 patients) The mean basal excretion, 9 3 mg, increased on the second corticotropin day to 13 9 mg ($P < 0\,05$) (maximum 28 5 mg, above 20 mg in 2 patients)

Comparison

120 I U of gelatine corticotropin causes smaller total excretions of steroids than 120 I U of zinc hydroxide corticotropin However, only the difference of the mean excretions of Porter Silber 17 OHCS on the second corticotropin day was significant ($P < 0\,05$) 120 I U of gelatine corticotropin increased the excretion of Porter Silber 17 OHCS to 52 3 mg and that of ketogenic 17 OHCS to 67 2 mg on the second corticotropin day, 60 I U of gelatine corticotropin caused a clearly smaller excretion of Porter

Silber 17 OHCS to 51 5 mg and that of ketogenic 17 OHCS to 72 5 mg on the second corticotropin day and the new type of zinc hydroxide corticotropin to 120 I U , to 61 9 mg and 81 9 mg, respectively These excretions do not differ significantly (P<0 05) Therefore the adrenocortical responses with 120 I U if new corticotropin and 40 I U of old corticotropin are nearly of the same size, as expected The old and new corticotropin used in recommended doses (ratio 1 3) have nearly equal clinical potency with a tendency to somewhat higher mean response with 120 I U of new corticotropin than with 40 I U old corticotropin The difference between these responses is not great when we take into account the individual biological variations of the adrenocortical reserves

4 *Gelatine corticotropin (Depo ACTH®)* (Table 7, 14, 15)

a Porter Silber 17-OHCS

120 I U (19 patients) The mean basal excretion, 14 6 mg, increased on the first corticotropin day to 35 4 mg, on the second corticotropin day to 52 3 mg (P < 0 001) (maximum to 92 0 mg, above 50 mg in 10 patients) and decreased on the following day to 19 4 mg

Table 7

Excretion of 17 KS Porter Silber 17 OHCS and Ketogenic 17 OHCS (mg/24 hours) in two day ACTH Test (I U ACTH Twice Daily i m) *Gelatine ACTH (Depo ACTH®)* Mean ±SEM

I U	17 KS mg/24 hrs				Porter Silber 17 OHCS mg/24 hrs				Ketogenic 17 OHCS mg/24 hrs			
	C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
120 I U 13 M + 6 F = (19)	9 0 ±0 9 (18)	16 4 ±1 4 (19)	19 2 ±1 6 (19)	12 5 ±1 1 (18)	14 6 ±1 0 (18)	35 4 ±2 4 (19)	52 3 ±3 9 (19)	19 4 ±2 4 (18)	27 7 ±2 0 (18)	55 0 ±3 5 (19)	67 2 ±6 1 (19)	40 8 ±4 4 (18)
60 I U 6 M + 3 F = (9)	10 8 ±1 3 (9)	15 3 ±1 9 (9)	18 1 ±1 6 (9)	14 6 +1 6 (9)	10 9 ±1 6 (9)	22 7 ±2 5 (9)	33 7 ±2 4 (9)	13.2 ±2 5 (9)	30 7 ±4 6 (9)	40 7 ±6 5 (9)	61 0 +6 8 (9)	38 7 ±4 6 (9)
20 I U 9 M + 4 F = (13)	9 3 ±1 1 (13)	12 3 ±1 7 (13)	13 9 ±2 2 (13)	11 2 ±2 1 (13)	11 2 ±1 3 (13)	22 8 ±2 4 (13)	29 3 ±3 7 (13)	15 9 ±3 5 (13)	30 1 ±3 4 (13)	45 9 ±5 0 (13)	52 9 ±7 3 (13)	42 3 ±5 4 (13)

C=Control, I=ACTH, II=ACTH Fo=Following day
F=Female, M=Male

60 I U (9 patients) The mean basal excretion increased from 10 9 mg to 33 7 mg ($P < 0001$) on the second corticotropin day (maximum 51 6 mg, above 25 mg in 10 patients)

20 I U (13 patients) The mean basal excretion 11 2 mg, increased on the second corticotropin day to 29 3 mg ($P < 0001$) (maximum 51 6 mg, above 25 mg in 10 patients)

b Ketogenic 17 OHCS

120 I U (19 patients) The mean basal excretion 27 mg, increased on the first corticotropin day to 55 0 mg, on the second corticotropin day to 67 2 mg ($P < 0001$) (maximum 104 1 mg, above 75 mg in 8 patients) and decreased on the following day to 40 8 mg

60 I U (9 patients) The mean basal excretion increased from 30 7 mg to 61 9 mg ($P < 001$) on the second corticotropin day (maximum 87 0 mg above 75 mg in 3 patients)

20 I U (13 patients) The mean basal excretion of 30 1 mg increased on the second corticotropin day to 52 9 mg ($P < 001$) (maximum 115 0 mg, above 50 mg in 6 patients)

c 17 Ketosteroids

120 I U (19 patients) The mean basal excretion, 9 0 mg increased on the first corticotropin day to 16 4 mg, on the second corticotropin day to 19 2 mg ($P < 0001$) (maximum was 30 0 mg, above 20 mg in 8 patients) and decreased on the following day to 12 5 mg

60 I U (13 patients) The mean basal excretion increased from 10 8 mg on the second corticotropin day to 18 1 mg ($P < 001$) (maximum 26 5 mg above 20 mg in 2 patients)

20 I U (13 patients) The mean basal excretion 9 3 mg increased on the second corticotropin day to 13 9 mg ($P < 005$) (maximum 28 5 mg, above 20 mg in 2 patients)

Comparison

120 I U of gelatine corticotropin causes smaller total excretions of steroids than 120 I U of zinc hydroxide corticotropin However, only the difference of the mean excretions of Porter Silber 17 OHCS on the second corticotropin day was significant ($P < 005$) 120 I U of gelatine corticotropin increased the excretion of Porter Silber 17 OHCS to 52 3 mg and that of ketogenic 17 OHCS to 67 2 mg on the second corticotropin-day, 60 I U of gelatine corticotropin caused a clearly smaller excretion of Porter

Silber 17 OHCS than 120 I U, while the excretions of ketogenic 17 OHCS and 17 KS were nearly of the same size with 60 I U as with 120 I U, 20 I U of gelatine corticotropin increased the excretions of Porter Silber 17 OHCS and ketogenic 17 OHCS (29 3 and 52 9 mg respectively) nearly to the same level on the second corticotropin day as 60 I U, while the excretion of 17 KS was smaller with 20 I U (13 9 mg) than with 60 I U (18 1 mg)

C INDIVIDUAL EXCRETIONS OF PORTER SILBER 17-OHCS, KETOGENIC 17 OHCS AND 17-KS FOR DIFFERENT LONG ACTING CORTICOTROPIN PREPARATIONS IN THE TWO DAY CORTICOTROPIN TEST

The individual excretions are grouped for each corticotropin dose according to the intensity of excretion of ketogenic 17 OHCS during the second corticotropin day (Table 8—15)

1 *Polyphloretin phosphate corticotropin* (Table 8 and 9)

On the second corticotropin day the individual excretions ranged widely with 60 I U 23 8—112 0 mg for Porter Silber 17 OHCS, 43 0—170 0 mg for ketogenic 17 OHCS and 8 5—46 5 mg for 17 KS With 40 I U the ranges were 23 1—97 0 mg, 40 0—120 0 mg and 13 5—30 0 mg respectively 60 I U as well as 40 I U increased the excretion of Porter Silber and ketogenic 17 OHCS clearly in each individual patient except one Also the excretion of 17 KS increased quite regularly except in seven patients after 60 I U

The increases of steroid excretions were clearly smaller with 20 I U, although the clear increase was observed in the majority of these patients The ranges varied 11 7—44 8 mg Porter Silber 17 OHCS, 13 0—102 0 mg ketogenic 17 OHCS and 1 9—17 5 mg 17 KS The excretion of Porter Silber 17 OHCS was not increased in one patient and that of ketogenic 17 OHCS slightly increased in one patient The excretion of 17 KS was not increased in three patients and only very slightly in three other patients

2 *Carboxymethyl cellulose corticotropin* (Table 10)

With 60 I U the ranges of excretions on the second corticotropin day were 14 3—106 1 mg for Porter Silber 17 OHCS, 28 0—142 0 mg for ketogenic 17 OHCS and 6 5—30 5 mg for 17 KS and with 20 I U 18 5—66 6 mg 10 0—125 0 mg and 15 5—31 5 mg respectively 60 I U increased clearly the steroid excretions except that of Porter Silber 17 OHCS and ketogenic 17 OHCS in one patient and that of 17 KS in two patients

20 I U also increased the excretion of these steroids, but not that of Porter Silber 17 OHCS and ketogenic 17 OHCS in one patient 17 KS also increased only very slightly in two patients Sometimes 20 I U caused equal increase of steroid excretion than 60 I U

3 *Zinc hydroxide corticotropin* (Table 11, 12 and 13)

The ranges of the individual excretions ranged widely On the second corticotropin-day with 120 I U of new type of corticotropin they were 9 0—117 8 mg for Porter Silber 17 OHCS, 16 0—165 0 mg for ketogenic 17 OHCS and 6 9—74 5 mg for 17 KS with 40 I U 9 8—58 8 mg 25 0—110 0 mg and 7 0—78 5 mg, respectively

With 120 I U of new type of corticotropin the excretions of Porter Silber 17 OHCS and ketogenic 17 OHCS were nearly always increased, except the excretion of Porter Silber 17 OHCS in one patient and that of ketogenic 17 OHCS in two patients The excretion of 17 KS also nearly always increased, although in three patients only slightly

On the other hand 40 I U of new type of corticotropin showed the smaller increase than 120 I U The excretion of Porter-Silber 17 OHCS was not or only very slightly increased in four patients, ketogenic 17 OHCS slightly increased in two patients and 17 KS not increased in two patients 20 I U caused somewhat greater response for Porter Silber 17 OHCS than 40 I U in a great number of patients

With 40 I U of old type of corticotropin the steroid excretions were almost always increased but not in one patient for Porter Silber 17 OHCS also in one patient for ketogenic 17 OHCS and very slightly increased in two patients for 17 KS In the majority of these patients 40 I U of old type caused nearly equal increase of steroid excretion than 120 I U of new type

4 *Gelatine corticotropin* (Table 14 and 15)

On the second corticotropin day the ranges of the individual excretion were With 120 I U 27 4—92 0 mg for Porter Silber 17 OHCS 25 0—104 1 mg for ketogenic 17 OHCS and 4 0—30 0 mg for 17 KS With 60 I U they were 18 5—44 2 mg 30 0—67 0 mg and 9 5—26 5 mg and with 20 I U 8 4—51 6 mg 26 0—115 0 mg and 4 9—28 5 mg respectively

120 I U increased clearly the steroid excretions except that of ketogenic 17 OHCS and 17 KS only very slightly in one patient

20 I U increased the steroid excretions nearly to the same level as 60 I U but in the majority of patients clearly smaller than 120 I U With 60 I U the excretion of Porter Silber 17 OHCS was not increased in one

Silber 17 OHCS than 120 I U, while the excretions of ketogenic 17 OHCS and 17 KS were nearly of the same size with 60 I U as with 120 I U, 20 I U of gelatine corticotropin increased the excretions of Porter-Silber 17 OHCS and ketogenic 17 OHCS (29 3 and 52 9 mg respectively) nearly to the same level on the second corticotropin day as 60 I U, while the excretion of 17 KS was smaller with 20 I U (13 9 mg) than with 60 I U (18 1 mg)

C INDIVIDUAL EXCRETIONS OF PORTER SILBER 17-OHCS, KETOGENIC 17 OHCS AND 17-KS FOR DIFFERENT LONG ACTING CORTICOTROPIN PREPARATIONS IN THE TWO DAY CORTICOTROPIN TEST

The individual excretions are grouped for each corticotropin dose according to the intensity of excretion of ketogenic 17 OHCS during the second corticotropin day (Table 8—15)

1 *Polyphloretin phosphate corticotropin* (Table 8 and 9)

On the second corticotropin day the individual excretions ranged widely with 60 I U 23 8—112 0 mg for Porter Silber 17-OHCS, 43 0—170 0 mg for ketogenic 17 OHCS and 8 5—46 5 mg for 17 KS With 40 I U the ranges were 23 1—97 0 mg, 40 0—120 0 mg and 13 5—30 0 mg, respectively 60 I U as well as 40 I U increased the excretion of Porter Silber and ketogenic 17 OHCS clearly in each individual patient, except one Also the excretion of 17 KS increased quite regularly, except in seven patients after 60 I U

The increases of steroid excretions were clearly smaller with 20 I U, although the clear increase was observed in the majority of these patients The ranges varied 11 7—44 8 mg Porter Silber 17 OHCS, 13 0—102 0 mg ketogenic 17 OHCS and 1 9—17 5 mg 17 KS The excretion of Porter Silber 17 OHCS was not increased in one patient and that of ketogenic 17 OHCS slightly increased in one patient The excretion of 17 KS was not increased in three patients and only very slightly in three other patients

2 *Carboxymethyl cellulose corticotropin* (Table 10)

With 60 I U the ranges of excretions on the second corticotropin day were 14 3—106 1 mg for Porter Silber 17 OHCS 28 0—142 0 mg for ketogenic 17 OHCS and 6 5—30 5 mg for 17 KS and with 20 I U 18 5—66 6 mg, 10 0—125 0 mg and 15 5—31 5 mg, respectively 60 I U increased clearly the steroid excretions except that of Porter Silber 17 OHCS and ketogenic 17 OHCS in one patient and that of 17 KS in two patients

40 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS				Ketogenic 17 OHCS				17 KS			
				C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
1 Amitriptyline 75 mg	1	F	44	11.8	40.3	83.2	31.2	32.0	60.0	120.0	63.0	17.0	20.5	23.0	32.0
2 —	2	M	45	20.4	24.9	97.0	14.7	40.0	42.0	115.0	49.0	9.5	10.0	17.5	17.0
3 —	3	M	55	14.3	39.0	61.2	57.0	38.0	91.0	112.0	[illegible]	9.0	13.0	15.0	12.0
4 —	4	M	18	20.8	[illegible]	71.0	22.0	37.0	117.0	110.0	33.0	21.5	27.0	30.0	19.0
5 Diphenylhydantoin 300 mg	5	F	39	27.3	61.0	[illegible]	38.4	31.0	53.0	103.0	73.0	5.5	11.0	21.5	-0.5
6 —	6	M	41	14.3	75.0	88.0	32.9	35.0	118.0	105.0	66.0	14.0	31.5	28.0	25.5
7 —	7	F	15	8.1	17.6	55.0	23.5	23.0	59.0	104.0	41.0	9.0	22.5	26.0	18.5
8 —	8	F	49	4.6	30.8	70.0	26.4	13.0	61.0	101.0	45.0	5.0	11.5	21.5	11.5
9 —	9	F	43	16.3	25.9	62.8	47.0	17.0	28.0	86.0	73.0	8.0	11.5	16.5	15.0
10 —	10	M	56	14.8	[illegible]	35.7	44.9	45.0	34.0	66.0	84.0	12.5	9.0	20.0	16.5
11 —	11	F	16	4.6	25.2	52.5	13.4	14.0	40.0	64.0	39.0	6.5	16.0	25.0	13.0
12 Diazepam 5 mg	12	M	57	7.6	18.9	46.3	11.2	35.0	50.0	82.0	33.0	11.5	11.5	21.0	17.0
13 —	13	F	62	9.9	34.0	78.0	32.1	36.0	50.0	77.0	56.0	16.0	13.0	20.0	12.5
14 Primidone 500 mg, Diphenylhydantoin 300 mg	14	F	24	9.2	77.4	73.2	14.9	9.8	51.0	75.0	20.0	3.0	10.0	13.5	6.5
15 —	15	M	52	12.0	20.5	36.7	16.8	21.0	27.0	70.0	36.0	7.5	11.5	22.0	17.0
16 —	16	F	29	9.2	49.9	65.0	32.0	12.0	48.0	63.0	49.0	9.5	12.5	18.0	13.5
17 Diphenylhydantoin 400 mg	17	M	41	12.0	10.3	23.1	7.7	17.0	23.0	60.0	10.8	7.3	9.5	22.0	6.0
18 —	18	F	32	12.5	46.8	44.9	41.6	32.0	49.0	40.0	32.0	5.5	10.5	16.0	15.0

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

Table 8

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 60 I U and 40 I U *polyphloretin phosphate corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the *following* day

60 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 —	1	M	52	13.5	33.9	92.8	6.0	29.0	65.0	170.0	31.0	12.0	—	42.0	9.5
2 —	2	F	39	10.7	45.2	93.4	48.0	15.0	70.0	149.0	71.0	9.5	13.5	26.5	22.0
3 —	3	F	54	11.2	42.2	72.3	38.5	50.0	84.0	136.0	73.0	13.0	24.0	46.5	29.0
4 —	4	M	37	27.0	27.3	88.0	26.4	54.0	41.0	122.0	53.0	20.0	14.0	33.0	12.0
5 —	5	M	63	7.7	25.2	36.1	40.0	17.0	51.0	72.0	105.0	7.0	17.0	28.0	37.0
6 —	6	M	39	27.6	37.9	46.2	24.8	43.0	63.0	85.0	45.0	15.5	16.5	26.5	19.5
7 Diphenylhydantoin 100 mg	7	M	44	25.2	47.5	59.0	22.5	36.0	74.0	85.0	40.0	6.5	13.5	14.0	8.0
8 —	8	M	58	4.1	36.9	64.8	19.5	15.0	83.0	79.0	59.0	11.0	12.0	20.0	15.0
9 —	9	F	44	28.2	108.0	80.4	32.8	22.0	118.0	79.0	42.0	0.9	22.0	21.0	15.0
10 *Diphenylhydantoin 300 mg*	*10*	F	*37*	*19.7*	*62.1*	*72.8*	*21.4*	*21.0*	*83.0*	*77.0*	45.0	*8.1*	*17.0*	*20.5*	*13.0*
11 —	11	M	43	19.3	42.3	51.6	37.7	44.0	95.0	75.0	64.0	13.5	29.0	24.0	16.5
12 —	12	M	49	11.0	60.3	86.4	28.2	21.0	102.0	68.0	59.0	13.0	26.0	29.5	21.5
13 —	13	M	57	11.1	34.8	70.0	—	16.8	37.0	68.0	—	6.0	5.5	9.0	—
14 —	14	F	64	20.0	15.7	—	31.7	68.0	44.0	—	65.0	13.0	9.5	—	27.0
15 —	15	M	53	13.2	24.9	48.9	17.3	13.0	50.0	60.0	52.0	16.0	18.5	18.5	15.0
16 —	16	M	34	8.3	16.8	23.8	27.1	15.0	41.0	52.0	48.0	6.5	10.5	8.5	14.0
17 Diphenylhydantoin 300 mg	17	F	42	37.5	101.7	112.0	32.8	22.0	54.0	43.0	22.0	9.5	13.5	13.0	12.5
18 —	18	M	36	20.7	39.9	42.0	22.0	29.0	59.0	43.0	18.0	8.5	21.0	16.5	14.0
19 —	19	M	31	19.2	25.6	—	—	55.0	52.0	—	—	17.5	17.5	—	—

40 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 Amitriptyline 75 mg	1	F	44	11.9	40.3	83.2	31.2	32.0	60.0	1_0.0	63.0	17.0	20.5	23.0	32.0
2	2	M	45	20.4	24.9	97.0	44.7	40.0	42.0	115.0	49.0	9.5	10.0	17.5	17.0
3	3	M	55	14.3	39.0	61.2	57.6	39.0	91.0	112.0	78.0	9.0	13.0	15.0	12.0
4 —	4	M	18	20.8	72.4	71.0	27.0	37.0	117.0	110.0	33.0	21.5	27.0	30.0	19.0
5 Diphenylhydantoin 300 mg	5	F	39	27.3	61.0	76.0	39.4	31.0	55.0	105.0	73.0	5.5	11.0	21.5	20.5
6 —	6	M	41	14.3	75.0	89.0	32.8	35.0	119.0	105.0	66.0	14.0	31.5	28.0	25.5
7 —	7	F	15	8.1	17.6	53.0	23.5	23.0	59.0	104.0	41.0	9.0	22.5	26.0	18.5
8 —	8	F	48	4.6	70.8	70.0	26.4	13.0	61.0	101.0	45.0	5.0	11.5	21.5	11.5
9 —	9	F	43	10.3	25.9	62.8	47.6	17.0	23.0	86.0	73.0	8.0	11.5	16.5	15.0
10 —	10	M	50	14.8	21.1	33.7	44.8	45.0	34.0	66.0	84.0	12.5	9.0	20.0	16.5
11 —	11	I	16	4.0	25.2	52.5	13.4	14.0	40.0	84.0	39.0	6.5	10.0	25.0	13.0
12 Diazepam 5 mg	12	M	57	7.6	18.9	46.3	11.2	35.0	50.0	82.0	33.0	11.5	11.5	21.0	17.0
13 —	13	F	62	9.9	34.0	78.0	32.1	36.0	50.0	77.0	56.0	10.0	13.0	20.0	12.5
14 Primidone 500 mg Diphenylhydantoin 300 mg	14	F	24	9.2	77.4	73.2	14.9	9.8	51.0	75.0	20.0	3.0	10.0	11.5	6.5
15 —	15	M	52	12.0	20.5	38.7	16.8	21.0	27.0	70.0	36.0	7.5	11.5	22.0	17.0
16 —	16	F	29	9.2	48.9	65.0	32.0	12.0	48.0	65.0	48.0	9.5	12.5	18.0	13.5
17 Diphenylhydantoin 400 mg	17	M	41	12.0	10.3	23.1	7.7	17.0	23.0	60.0	10.9	7.3	9.5	22.0	8.0
18 —	18	F	32	12.5	46.8	44.8	41.6	32.0	49.0	40.0	32.0	5.5	10.5	16.0	15.0

C = Control, I = ACTH, II = ACTH, Fo = Following day
F = Female, M = Male

Table 9

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 20 I U *polyphloretin phosphate corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

20 I U

Other Treatment Dose/day	No of Pat	Sex	Age Years	Porter Silber 17 OHCS C	Porter Silber 17 OHCS I	Porter Silber 17 OHCS II	Porter Silber 17 OHCS Fo	Ketogenic 17 OHCS C	Ketogenic 17 OHCS I	Ketogenic 17 OHCS II	Ketogenic 17 OHCS Fo	17 KS C	17 KS I	17 KS II	17 KS Fo
1 —	1	M	15	12.9	18.2	35.7	7.0	63.0	62.0	102.0	65.0	11.0	11.5	14.5	16.5
2 —	2	F	61	9.0	29.7	37.8	23.8	15.0	38.0	78.0	29.0	3.1	7.5	15.0	19.0
3 Diphenylhydantoin 400 mg, Diazepam 10 mg	3	M	45	10.7	19.4	25.3	3.1	49.0	63.0	59.0	19.0	8.0	11.5	16.0	4.5
4 Diazepam 10 mg	4	F	62	12.8	29.2	44.8	33.5	16.0	44.0	54.0	56.0	4.0	7.0	12.0	15.5
5 —	5	M	57	11.9	11.8	12.8	1.2	36.0	61.0	54.0	33.0	11.5	15.0	16.0	9.5
6 —	6	M	18	9.5	19.0	34.8	10.1	23.0	70.0	48.0	26.0	11.0	16.0	17.5	15.0
7 —	7	M	53	9.0	7.4	31.7	7.6	27.0	23.0	44.0	25.0	9.0	10.0	13.5	11.5
8 Carbamazepine 400 mg	8	F	72	7.9	21.6	36.8	21.0	15.0	21.0	38.0	15.0	2.7	4.3	6.4	4.4
9 —	9	M	43	6.7	14.3	15.2	2.7	—	38.0	37.0	42.0	9.0	11.0	8.0	8.0
10 Diphenylhydantoin 300 mg Primidone 750 mg	10	F	44	14.6	34.2	—	17.2	18.0	33.0	—	18.0	5.7	6.0	—	3.7
11 —	11	F	16	18.9	16.0	11.7	5.8	15.0	26.0	14.0	0	1.4	5.5	4.5	4.0
12 Primidone 750 mg	12	F	44	8.9	16.4	34.4	12.3	0	8.0	13.0	8.5	1.6	1.0	1.3	1.6

C=Control I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

patient, that of ketogenic 17 OHCS in two patients and of 17-KS in three patients 20 I U caused no response in one patient for Porter Silber 17 OHCS and also for ketogenic 17 OHCS and in five patients for 17 KS

5 *All corticotropin preparations*

The basal excretion of some of the steroids was low in 16 patients (Table 8, 40 I U, no 14, Table 9, no 11 and no 12, Table 10, 60 I U, no 3, 5 and 13, Table 11, no 3 and 41, Table 12, 40 I U, no 20, Table 13, no 12, 21, 24 and 28, Table 14, no 19, Table 15, 60 I U, no 6 and 20 I U, no 13) Three of them (Table 8, 40 I U, no 14, Table 10, 60 I U, no 13, Table 11, no 3) were thin and their constitution was asthenic 9 patients of them were receiving some medical treatment during the corticotropin test There were no other differences

In 16 patients, on the other hand, the basal excretion of some of the steroids was high (Table 8, 60 I U, no 3, 4, 14 and 19, Table 8, 40 I U, no 4, Table 9 no 1, Table 10, 20 I U, no 8, Table 11, no 13, 18 and 26, Table 12, 40 I U, no 9 and 12, Table 12 20 I U, no 1, Table 13, no 6, Table 15, 60 I U, no 2, Table 15, 20 I U, no 2) In 4 patients (Table 8, 60 I U, no 4, Table 10, 20 I U, no 8, Table 11, no 13 and 18) the two day metopirone test was carried out 4—5 days earlier In one patient (Table 15 20 I U, no 2) the vertebral artery angiography and in other patient (Table 15 60 I U, no 2) the pneumoencephalography was performed 3 and 6 days earlier, respectively There were no other possible stress factors in these patients

Table 10

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i.m 60 I U and 20 I U *carboxymethyl cellulose corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

60 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS				Ketogenic 17 OHCS				17 KS			
				C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
1 —	1	F	48	12.0	35.8	68.0	10.6	28.0	66.0	142.0	39.0	9.0	16.0	28.0	16.0
2 —	2	F	57	7.9	50.9	103.7	44.0	12.0	52.0	126.0	39.0	9.5	19.0	30.0	18.5
3 Benztropine methanesulphomate 3 mg	3	F	41	1.0	23.8	41.7	11.2	21.0	47.0	88.0	37.0	0.4	9.0	15.5	7.0
4 —	4	M	57	8.1	25.6	15.9	10.8	22.0	51.0	83.0	39.0	7.0	12.5	18.5	11.0
5 —	5	F	20	2.5	45.8	106.1	78.1	7.4	66.0	50.0	79.0	7.0	23.0	15.0	20.5
6 —	6	F	37	6.8	40.0	40.4	35.7	13.0	72.0	43.0	79.0	3.6	13.2	13.0	18.0
7 —	7	F	30	3.6	29.6	54.0	24.8	15.0	77.0	78.0	63.0	10.0	24.0	30.5	28.0
8 —	8	F	50	12.5	59.6	52.8	34.4	17.8	60.0	66.0	45.0	7.0	14.0	17.0	12.5
9 —	9	F	27	8.7	50.0	71.8	24.9	20.0	75.0	65.0	34.0	6.9	26.5	29.5	18.0
10 Belladonna 0.75 mg, Phenobarbital 60 mg	10	M	31	16.8	27.1	14.3	26.4	42.0	54.0	58.0	34.0	9.0	9.5	6.5	9.0
11 —	11	F	55	3.4	22.6	60.1	20.0	25.0	37.0	53.0	27.0	6.0	7.0	16.5	19.0
12 —	12	F	28	12.0	34.9	55.4	47.0	18.0	32.0	50.0	21.0	8.0	10.5	30.5	12.5
13 Primidone 1 g, Diphenylhydantoin 200 mg Phenobarbital 100 mg	13	F	63	18.0	39.0	62.9	19.1	22.0	40.0	41.0	21.0	1.9	7.0	7.0	6.5
14 —	14	F	59	5.3	11.9	28.5	40.6	28.0	22.0	28.0	37.0	8.5	4.5	9.5	15.0
15 Diphenylhydantoin 200 mg Phenobarbital 100 mg Primidone 250 mg	15	F	47	6.2	34.1	52.3	12.0	17.0	29.0	36.0	21.0	6.5	10.5	12.5	12.5

40 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 *Diazepam 20 mg*	1	M	47	19.0	29.7	53.2	21.3	72.0	115.0	125.0	55.0	13.5	24.0	20.0	9.5
2 —	2	M	54	13.2	13.8	55.0	16.1	46.0	77.0	111.0	40.0	11.5	13.0	19.0	12.0
3 —	3	M	30	12.0	32.9	49.6	21.1	47.0	79.0	93.0	66.0	13.5	22.5	21.5	14.5
4 —	4	F	21	18.5	31.2	47.0	30.3	38.0	81.0	95.0	63.0	9.0	16.5	22.5	15.5
5 —	5	M	64	12.0	8.0	60.6	16.6	24.0	10.0	74.0	20.0	5.7	3.2	15.5	5.0
6 —	6	M	42	8.3	17.2	23.6	21.1	32.0	55.0	55.0	63.0	10.0	32.5	31.5	19.0
7 —	7	M	51	5.2	26.1	37.2	8.2	36.0	56.0	63.0	38.0	—	16.5	17.0	15.5
8 —	8	M	35	12.6	13.5	18.5	4.8	59.0	44.0	60.0	39.0	16.5	10.5	21.0	13.5
9 —	9	M	50	6.7	21.0	29.3	10.4	27.0	50.0	45.0	55.0	16.0	22.5	20.0	17.0
10 Perphenazine 16 mg	10	M	43	7.2	26.4	26.0	39.6	17.0	26.0	10.0	53.0	11.0	30.0	23.0	28.5

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

Table 11

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 120 I U *zinc hydroxide corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

120 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 —	1	F	39	5.7	4.3	30.1	80.0	25.0	20.0	105.0	200.0	11.5	5.5	26.5	27.5
2 —	2	F	30	2.2	17.0	102.0	117.0	42.0	40.0	146.0	170.0	7.5	3.5	16.0	20.5
3 —	3	F	32	2.9	45.1	76.6	77.1	6.6	66.0	75.0	168.0	2.0	10.5	25.0	30.0
4 —	4	M	39	2.5	23.4	70.6	61.2	42.0	85.0	165.0	131.0	9.5	18.5	24.0	34.0
5 —	5	M	52	7.6	72.8	105.0	124.6	32.0	82.0	65.0	155.0	14.0	49.5	74.5	100.0
6 —	6	F	51	3.0	13.7	46.8	69.4	38.0	32.0	88.0	146.0	8.0	4.5	13.0	20.0
7 —	7	M	31	15.5	33.2	66.0	69.1	24.0	69.0	124.0	141.0	4.0	7.5	7.0	8.5
8 —	8	F	53	5.5	32.1	80.0	67.2	37.0	62.0	130.0	96.0	6.5	11.5	30.0	30.5
9 —	9	F	30	16.9	51.0	70.8	101.9	28.0	77.0	93.0	128.0	9.0	17.5	19.0	46.0
10 —	10	M	57	9.9	40.1	117.8	126.5	27.0	61.0	124.0	93.0	9.0	18.5	34.5	12.5
11 —	11	M	48	20.7	43.7	77.8	107.9	50.0	72.0	75.0	123.0	6.5	13.5	31.0	42.0
12 —	12	M	41	7.7	27.6	65.6	74.4	20.0	57.0	118.0	101.0	5.5	14.0	22.5	31.5
13 —	13	M	48	4.2	31.7	67.2	25.2	35.0	72.0	118.0	77.0	17.9	17.5	38.5	25.0
14 —	14	M	31	16.2	42.6	85.8	77.4	36.0	61.0	100.0	110.2	17.5	29.5	40.5	36.0
15 —	15	F	49	9.6	37.0	75.6	70.0	36.0	56.0	106.0	91.0	16.5	14.5	30.0	26.0
16 Diphenylhydantoin 300 mg	16	F	40	14.3	59.4	100.0	102.6	18.0	44.0	102.0	106.0	5.7	9.0	19.0	26.5
17 —	17	F	53	17.0	49.0	52.6	74.5	22.0	56.0	74.0	105.0	3.3	6.0	9.5	12.5
18 —	18	M	46	7.4	40.0	88.0	67.2	57.0	62.0	104.0	87.0	6.0	18.0	45.5	52.5
19 —	19	F	46	8.0	68.5	108.0	98.8	29.0	52.0	98.0	85.0	5.0	16.5	27.0	29.5
20 —	20	M	38	1.8	13.7	29.0	18.6	24.0	65.0	98.0	70.0	11.0	13.5	12.5	22.0

21 —	21	F	40	6.4	20.4	46.7	11.1	37.0	62.0	94.0	49.0	10.0	13.0	23.0	16.5
22 —	22	M	32	11.3	8.7	9.0	19.6	49.5	72.0	90.0	74.0	13.5	11.0	13.0	16.0
23 —	23	F	43	3.1	26.8	67.6	30.4	29.0	79.0	97.0	69.0	4.0	11.5	12.5	13.5
24 —	24	F	61	3.5	6.6	48.0	58.0	15.0	24.0	62.0	85.0	3.1	5.5	10.1	23.0
25 —	25	F	43	22.9	42.0	38.4	61.6	20.0	40.0	55.0	85.0	4.5	13.0	15.5	11.5
26 Thioridazine *100 mg*, Belladonna *0.60 mg*, *Phenobarbital 100 mg*	26	M	29	46.4	34.2	68.5	66.9	48.0	55.0	83.0	62.0	9.0	9.0	13.5	20.0
27 —	27	F	37	4.0	19.8	73.1	93.5	36.0	38.0	80.0	64.0	10.5	10.0	45.0	38.0
28 —	28	F	35	7.4	19.4	61.6	60.5	25.0	39.0	78.0	74.0	4.9	7.0	13.0	19.0
29 —	29	F	33	15.4	42.3	63.4	71.1	33.0	57.0	69.0	78.0	11.0	20.5	25.5	34.0
30 —	30	F	38	10.7	20.5	45.6	56.6	22.0	24.0	59.0	78.0	3.7	4.2	6.9	11.0
31 —	31	M	52	2.1	33.0	70.0	63.0	32.0	60.0	73.0	62.0	6.5	6.5	14.5	22.0
32 —	32	M	29	9.4	30.4	70.5	80.5	24.0	69.0	72.0	58.0	7.0	21.5	52.5	59.0
33 —	33	F	26	7.3	17.8	22.6	86.8	40.0	63.0	70.0	68.0	15.0	38.5	38.0	40.5
34 —	34	F	47	4.7	37.8	44.2	30.8	12.0	69.0	62.0	69.0	8.0	11.0	14.0	24.5
35 —	35	F	43	7.6	25.0	40.0	46.2	28.0	42.0	59.0	69.0	11.0	12.0	15.0	21.5
36 —	36	M	32	13.8	8.8	16.8	21.2	60.0	50.0	54.0	69.0	10.5	9.0	13.5	28.5
37 —	37	F	46	9.1	40.5	72.0	37.2	15.6	49.0	62.0	61.0	10.5	18.5	27.5	30.5
38 Neostigmine *60 mg*, Pyridostigmine *20 mg*	38	F	20	3.7	10.9	49.6	46.8	30.0	34.0	62.0	50.0	7.9	8.3	24.0	25.0
39 —	39	F	55	24.3	36.9	32.0	45.2	40.0	33.0	74.0	59.0	18.0	17.0	17.0	21.5
40 —	40	F	42	8.3	32.8	41.4	36.0	28.0	53.0	47.0	46.0	5.3	11.5	14.0	15.5
41 —	41	F	33	7.8	50.2	76.0	54.6	7.2	40.0	46.0	39.0	4.5	10.5	17.5	9.5
42 —	42	M	33	10.1	12.7	21.9	10.1	12.0	25.0	44.0	29.0	9.0	7.0	15.5	20.5
43 —	43	M	34	2.7	26.2	63.0	61.2	11.0	41.0	39.0	42.0	7.0	7.5	21.0	24.0
44 —	44	F	46	7.2	26.5	59.4	55.0	41.0	31.0	16.0	22.0	6.2	11.0	20.0	26.5

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

Table 12

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 40 I U and 20 I U *zinc hydroxide corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

40 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 Papaverine 300 mg	1	M	60	4.5	31.9	57.6	42.5	27.0	86.0	110.0	100.0	4.8	14.0	22.0	21.5
2 —	2	M	43	8.6	18.5	38.4	19,1	37.0	39.0	89.0	46.0	8.0	7.0	18,5	16.5
3 Amitriptyline 150 mg, Prochlorperazine 15 mg	3	M	51	11.2	17.0	23.9	31.0	50.0	51.0	66.0	79,0	14.5	13.5	21.0	25.0
4 —	4	F	30	6.7	35.2	58.8	54.0	27.0	48.0	55.0	73.0	12.0	18.5	20.5	25.5
5 —	5	M	73	15.9	21.6	43.6	35.4	28.0	34.0	72.0	47.0	4.5	7.5	21.5	10.5
6 Diphenylhydantoin 200 mg	6	M	15	5.2	24.0	32.2	36.8	19.0	53.0	60.0	63.0	5.9	16.5	24.0	33.0
7 —	7	M	53	3.1	11.8	44.6	61.9	20.0	40.0	54.0	63.0	6.8	18.5	21.0	29.5
8 —	8	F	36	2.7	14.3	48.0	46.8	15.0	23.0	52.0	62.0	9.0	20.5	17.5	23.0
9 —	9	F	35	7.1	31.9	36.6	33.6	16.0	49.0	61.0	44.0	20.5	44.5	78.5	58.0
10	10	F	33	11.0	27.3	44.5	36.0	33.0	69.0	59.0	52.0	5.3	8.5	11.5	11.5
11 —	11	M	53	16.1	16.6	18.0	12.4	42.0	50.0	59.0	49.0	5.6	8.5	10.5	10.0
12 —	12	M	49	10.6	34.3	13.2	25.0	76.0	83.0	43.0	58.0	16.5	22.5	19.5	20.0
13 Diazepam 10 mg	13	M	56	9.8	16.0	11.5	36.0	16.0	17.0	35.0	58.0	6.5	13.0	12.5	21.0
14 —	14	F	37	20.5	24.2	27.8	16.4	40.0	44.0	51.0	36.0	7.0	8.2	7.0	5.5
15 —	15	F	40	20.6	24.3	54.4	35.4	23.0	32.0	50.6	45.0	10.0	12.0	23.5	24.0
16 —	16	F	17	8.9	11.5	11.2	2.6	27.0	26.0	36.0	21.0	3.4	5.7	7.5	4.1
17 —	17	F	60	7.7	26.3	37.0	24.5	10.0	25.0	35.0	32.0	4.8	8.0	14.0	13.0
18 —	18	M	39	13.4	11.6	9.8	18.5	18.0	28.0	25.0	33.0	5.7	13.0	13.5	25.0
19 Reserpine 0.5 mg	19	F	41	8.8	33.0	31.9	25.9	21.0	29.0	33.0	22.0	9.5	13.5	17.5	16.6
20 —	20	F	61	11.7	39.0	—	29.5	3.0	19.0	—	23.0	10.5	11.0	—	4.5

20 IU

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 —	1	M	49	10.2	30.7	78.0	29.5	54.0	50.0	118.0	118.0	16.0	18.0	30.5	40.0
2 —	2	M	21	12.9	31.1	74.8	30.7	31.0	60.0	101.0	76.0	13.0	10.5	32.0	21.5
3 —	3	F	40	10.7	43.8	53.2	51.9	18.0	82.0	83.0	94.0	6.0	13.0	19.0	19.5
4 —	4	M	49	15.0	63.2	50.0	58.6	36.0	74.0	32.0	94.0	5.5	13.0	11.5	19.0
5 —	5	F	62	16.4	38.4	51.3	31.1	34.0	50.0	86.0	74.0	11.5	21.5	29.0	23.5
6 —	6	M	39	8.1	30.2	32.8	42.2	38.0	74.0	72.0	86.0	5.5	19.0	22.0	21.5
7 —	7	M	16	19.7	42.7	60.0	43.2	50.0	73.0	47.0	76.0	11.0	13.5	19.5	31.0
8 Diphenylhydantoin 400 mg	8	F	40	17.2	20.9	52.0	14.4	49.0	43.0	72.0	44.0	9.5	9.0	18.5	14.5
9 —	9	M	56	12.0	23.8	47.6	24.8	34.0	54.0	69.0	51.0	12.0	17.5	16.5	14.0
10 —	10	F	51	21.3	20.4	21.8	52.5	20.0	42.0	28.0	69.0	6.5	8.5	6.0	14.5
11 —	11	M	64	6.3	33.5	80.9	48.0	35.0	46.0	60.0	64.0	12.0	16.0	15.5	15.0
12 —	12	M	51	12.4	35.3	48.6	43.2	35.0	63.0	63.0	59.0	5.0	9.5	12.5	16.5
13 Carbamazepine 800 mg	13	M	18	22.8	54.0	63.0	66.3	35.0	49.0	60.0	40.0	10.0	10.5	8.0	10.0
14 —	14	M	47	14.3	55.2	90.8	26.0	32.0	70.0	47.0	55.0	17.5	28.5	31.5	27.0
15 —	15	F	32	5.6	16.5	27.0	21.8	26.0	46.0	33.0	33.0	9.0	11.5	13.5	14.0
16 —	16	M	18	6.6	19.7	29.3	18.9	23.0	40.0	31.0	30.0	10.0	15.0	16.0	19.0

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

Table 18

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 40 I U of *old type of zinc hydroxide corticotropin* twice daily In this series two control days are first and then two ACTH days Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

40 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS				Ketogenic 17 OHCS				17 KS			
				C	C	I	II	C	C	I	II	C	C	I	II
1 —	1	M	30	13.9	13.9	43.9	72.0	46.0	42.0	65.0	147.0	6.6	5.0	6.4	14.0
2 —	2	F	14	8.5	12.7	35.4	148.9	20.0	23.0	61.0	116.0	5.8	8.1	15.0	24.0
3 —	3	M	24	18.8	—	43.7	126.6	23.0	—	61.0	105.0	14.0	—	32.0	42.0
4 Prochlorperazine 75 mg	4	M	30	2.9	6.5	48.3	71.5	—	—	—	—	8.7	6.8	26.5	27.5
5 Belladonna 0.4 mg, Phenobarbital 80 mg	5	F	54	8.8	4.4	34.0	70.6	—	—	—	—	10.0	5.1	37.5	52.3
6 Imipramine 100 mg, Nialamide 150 mg, Thioridazine 50 mg	6	F	35	3.1	6.8	10.1	56.0	33.0	31.0	69.0	100.0	29.5	24.0	46.5	—
7 Chlorpromazine 300 mg	7	F	31	—	3.3	23.5	45.3	26.0	32.0	47.0	92.0	11.0	9.9	15.0	22.0
8 —	8	F	28	—	—	—	—	46.0	23.0	88.0	80.0	15.0	9.9	22.0	31.0
9 —	9	F	35	7.9	12.0	64.2	114.8	39.0	59.0	84.0	83.0	16.0	23.0	33.0	37.0
10 —	10	M	36	9.8	4.6	36.0	59.6	22.0	28.0	63.0	80.0	9.9	9.9	17.0	23.0
11 —	11	M	36	8.6	1.0	34.5	21.4	35.0	10.0	80.0	48.0	2.5	1.0	5.7	3.0
12 Nialamide 75 mg	12	M	36	7.4	3.5	32.0	51.0	—	—	—	—	1.6	2.3	4.8	2.9
13 Nialamide 75 mg	13	F	17	7.9	5.3	13.4	44.9	43.0	16.0	31.0	75.0	2.9	2.3	6.2	11.5
14 —	14	F	23	11.0	15.3	32.5	56.9	27.0	20.0	54.0	72.0	2.4	3.0	4.3	5.9
15 Chlorprothixene 60 mg	15	F	36	6.7	9.2	41.3	64.9	23.0	17.0	71.0	61.0	4.0	3.6	14.0	18.0
16 —	16	F	31	7.9	8.7	12.0	9.0	37.0	37.0	33.0	70.0	4.8	8.0	8.6	14.0

17 —	17	M	55	11.3	12.0	27.0	53.9	34.0	16.0	21.0	62.0	4.5	1.6	5.1	8.6
18 Meprobamate 400 mg	18	M	29	18.5	13.5	38.0	67.8	46.0	49.0	49.0	61.0	10.5	6.9	12.0	16.0
19 —	19	F	24	9.6	8.0	14.5	33.3	37.0	41.0	38.0	61.0	18.0	11.0	21.0	23.0
20 Belladonna 0.50 mg Phenobarbital 100 mg	20	M	33	4.0	2.7	6.7	31.9	27.0	23.0	25.0	57.0	0.5	5.8	5.1	12.0
21 Prochlorperazine 30 mg	21	M	15	4.5	8.4	18.8	32.9	4.0	18.0	27.0	54.0	0.9	12.0	12.1	25.6
22 Meprobamate 800 mg	22	F	37	17.2	21.4	32.5	38.7	32.0	20.0	41.0	52.0	3.7	3.1	5.8	7.3
23 Belladonna 0.50 mg Phenobarbital 100 mg	23	F	53	10.0	5.0	19.0	50.8	25.0	21.0	28.0	49.0	5.0	5.1	11.0	15.0
24 —	24	F	41	1.6	4.8	50.6	17.0	—	—	—	—	1.0	2.9	1.3	8.8
25 —	25	M	38	6.1	3.4	19.8	48.0	—	—	—	—	15.5	17.0	21.0	36.0
26 —	26	F	47	3.4	3.5	21.1	23.9	—	13.0	30.0	47.0	12.0	12.5	16.5	17.0
27 —	27	F	73	19.3	10.1	22.2	45.4	—	—	—	—	15.0	13.0	21.0	49.0
28 Reserpine 1 mg	28	M	56	1.3	1.1	20.8	45.0	—	—	—	—	1.3	2.3	4.7	13.0
29 Thioridazine 75 mg	29	F	29	6.1	22.9	31.7	41.8	—	—	—	—	9.5	13.0	21.5	19.0
30 —	30	F	44	1.6	—	25.0	39.5	—	—	—	—	3.0	—	7.1	10.0
31 Imipramine 75 mg	31	F	38	6.1	6.1	8.7	30.5	—	—	—	—	10.0	10.0	13.0	35.0
32 Chlorpromazine 300 mg	32	F	51	3.4	2.8	24.4	23.0	—	—	—	—	9.9	5.7	9.0	21.0
33 —	33	M	38	1.3	3.1	7.3	8.7	39.0	15.0	10.0	22.0	0.4	15.0	9.3	18.0

C = 2 Control days, I = ACTH, II = ACTH
F = Female, M = Male

Table 14

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 120 I U. *gelatine corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

120 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
1 —	1	M	57	7.8	31.5	92.0	33.0	35.0	41.0	104.1	83.0	8.0	13.5	17.0	19.5
2 —	2	M	47	16.4	37.4	57.1	6.4	25.0	75.0	100.0	30.0	16.0	22.5	28.5	9.5
3 —	3	M	18	10.0	31.5	68.6	22.5	39.0	67.0	96.0	63.0	7.0	17.5	21.5	17.0
4 —	4	F	21	—	32.6	44.4	13.7	—	38.0	91.0	36.0	—	15.5	22.0	11.5
5 —	5	F	30	19.5	43.5	72.0	18.0	26.0	66.0	90.0	33.0	7.5	15.5	23.5	15.5
6 Diphenylhydantoin 300 mg, Diazepam 10 mg	6	M	45	24.0	63.0	64.5	26.6	37.0	68.0	88.0	76.0	13.0	21.5	23.0	15.5
7 —	7	M	63	16.0	34.8	47.0	18.1	23.0	56.0	84.0	40.0	8.0	16.0	26.0	14.0
8 Diazepam 10 mg	8	M	55	16.3	35.0	55.7	15.4	32.0	51.0	82.0	35.0	11.0	9.5	15.0	14.5
9 —	9	F	23	17.2	35.2	50.5	21.1	34.0	68.0	74.0	37.0	12.5	27.0	29.0	18.0
10 —	10	M	48	11.6	19.0	69.1	22.0	21.0	55.0	67.0	45.0	7.0	19.0	17.0	9.0
11 —	11	M	64	13.4	39.3	36.1	13.7	37.0	50.0	65.0	51.0	9.5	14.5	15.0	6.0
12 Chlordiazepoxide 15 mg	12	M	34	7.6	22.9	43.5	13.2	31.0	49.0	61.0	25.0	10.0	21.0	30.0	17.0
13 Diphenylhydantoin 300 mg	13	F	52	14.8	30.1	36.8	18.7	21.0	53.0	55.0	31.0	7.5	11.0	12.0	10.0
14 Diphenylhydantoin 300 mg Diazepam 10 mg	14	M	40	14.5	24.0	27.5	12.2	33.0	47.0	48.0	35.0	8.0	15.0	19.0	10.5
15 —	15	F	52	15.6	47.2	41.6	—	20.0	83.0	47.0	—	10.0	17.0	15.5	—
16 —	16	M	32	13.9	23.9	27.4	20.6	27.0	50.0	39.0	41.0	13.5	25.0	19.0	17.5
17 Carbamazepine 600 mg Diphenylhydantoin 300 mg	17	M	16	11.2	29.4	44.1	26.1	14.0	33.0	34.0	24.0	3.0	10.5	10.5	7.0
18 —	18	M	42	15.5	44.0	64.4	4.9	35.0	67.0	29.0	32.0	10.0	19.5	17.5	9.5
19 Carbamazepine 800 mg	19	F	39	18.4	49.8	53.4	44.9	10.0	28.0	25.0	17.0	1.8	2.0	4.0	4.9

Table 15

Excretion of *Porter Silber* 17 OHCS *Ketogenic* 17 OHCS and 17 KS (mg/24 hours) *in* two day corticotropin test, i m 60 I U and 20 I U *gelatine corticotropin* twice daily Individual values grouped *according* to the *size of* the excretion *of ketogenic* 17 OHCS on the second ACTH day or on the *following* day

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS C	I	II	Fo	Ketogenic 17 OHCS C	I	II	Fo	17 KS C	I	II	Fo
60 I U															
1 —	1	M	33	7.5	14.8	44.2	26.0	31.0	47.0	87.0	53.0	13.0	12.0	19.0	20.5
2 —	2	M	52	20.0	30.1	41.4	9.2	54.0	62.0	83.0	53.0	8.5	12.5	17.0	18.0
3 —	3	M	27	10.2	23.0	24.6	14.8	33.0	68.0	82.0	47.0	14.7	22.5	23.0	15.0
4 —	4	M	42	12.5	36.9	36.9	21.8	19.0	49.0	66.0	54.0	15.0	27.0	26.5	21.0
5 —	5	F	49	11.2	20.9	38.4	5.0	38.0	49.0	58.0	36.0	12.5	15.0	13.0	11.0
6 Diphenylhydantoin 200 mg, Phenobarbital 100 mg	6	F	52	4.9	22.8	29.4	16.8	8.6	4.0	58.0	32.0	2.9	13.5	18.5	11.0
7 —	7	F	52	4.6	19.2	39.0	15.0	20.0	29.0	48.0	30.0	12.0	15.5	17.5	14.5
8 —	8	M	57	12.6	12.0	18.5	4.0	43.0	27.0	37.0	21.0	12.0	12.5	14.5	17.0
9 Diphenylhydantoin 300 mg	9	M	64	14.8	25.0	30.9	7.0	30.0	33.0	30.0	17.0	7.0	7.8	9.5	5.6
20 I U															
1 —	1	M	48	10.0	22.0	37.1	5.1	39.0	60.0	115.0	59.0	9.5	18.0	27.5	15.5
2 —	2	M	43	22.0	35.7	31.9	35.4	55.0	73.0	99.0	78.0	11.5	21.0	20.0	16.5
3 —	3	M	42	11.9	28.2	31.6	23.5	32.0	48.0	47.0	73.0	11.0	13.5	18.5	16.0
4 —	4	F	35	20.5	33.5	42.5	23.1	34.0	57.0	69.0	46.0	9.5	12.0	10.5	8.0
5 —	5	M	57	9.2	18.9	18.9	8.1	32.0	65.0	63.0	50.0	7.5	9.4	9.2	4.8
6 —	6	F	40	12.3	34.0	43.7	9.5	14.0	58.0	59.5	27.0	3.8	6.4	10.0	2.7
7 —	7	M	49	11.0	20.0	41.5	17.2	20.0	38.0	52.0	35.0	17.5	20.5	28.5	22.0
8 Chlordiazepoxide 15 mg	8	M	35	8.2	24.6	25.2	12.0	42.0	55.0	27.0	45.0	8.5	16.5	13.0	10.0
9 Diphenylhydantoin 300 mg	9	F	67	9.4	19.1	29.4	44.1	15.0	29.0	43.0	36.0	10.0	15.5	19.0	27.0
10 —	10	M	67	6.0	25.0	18.0	9.6	32.0	43.0	42.0	33.0	5.7	5.8	4.9	5.3
11 —	11	M	48	9.6	8.9	8.4	5.0	29.0	23.0	22.0	41.0	14.5	9.0	8.0	9.5
12 Diphenylhydantoin 300 mg	12	M	52	9.0	13.2	13.3	3.0	36.0	31.0	35.0	16.0	8.0	6.0	7.0	3.5
13 —	13	F	62	8.4	12.0	20.3	10.6	12.0	10.0	26.0	11.5	4.3	5.5	5.3	5.1

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

Table 14

Excretion of Porter Silber 17 OHCS, Ketogenic 17 OHCS and 17 KS (mg/24 hours) in two day corticotropin test, i m 120 I U *gelatine corticotropin* twice daily Individual values grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day

120 I U

Other Treatment Dose/day	No of pat	Sex	Age Years	Porter Silber 17 OHCS				Ketogenic 17 OHCS				17 KS			
				C	I	II	Fo	C	I	II	Fo	C	I	II	Fo
1 —	1	M	57	7.8	31.5	92.0	33.0	35.0	41.0	104.1	83.0	8.0	13.5	17.0	19.5
2 —	2	M	47	16.4	37.4	57.1	6.4	25.0	75.0	100.0	30.0	16.0	22.5	28.5	9.5
3 —	3	M	18	10.0	31.5	68.6	22.5	39.0	67.0	96.0	63.0	7.0	17.5	21.5	17.0
4 —	4	F	21	—	32.6	44.4	13.7	—	38.0	91.0	36.0	—	15.5	22.0	11.5
5 —	5	F	30	19.5	43.5	72.0	18.0	26.0	66.0	90.0	33.0	7.5	15.5	23.5	15.5
6 Diphenylhydantoin 300 mg, Diazepam 10 mg	6	M	45	24.0	63.0	64.5	26.6	37.0	68.0	88.0	76.0	13.0	21.5	23.0	15.5
7 —	7	M	63	16.0	34.8	47.0	18.1	23.0	56.0	84.0	40.0	8.0	16.0	26.0	14.0
8 Diazepam 10 mg	8	M	55	16.3	35.0	55.7	15.4	32.0	51.0	82.0	35.0	11.0	9.5	15.0	14.5
9 —	9	F	23	17.2	35.2	50.5	21.1	34.0	68.0	74.0	37.0	12.5	27.0	29.0	18.0
10 —	10	M	48	11.6	19.0	69.1	22.0	21.0	55.0	67.0	45.0	7.0	19.0	17.0	9.0
11 —	11	M	64	13.4	39.3	36.1	13.7	37.0	50.0	65.0	51.0	9.5	14.5	15.0	6.0
12 Chlordiazepoxide 15 mg	12	M	34	7.6	22.9	43.5	13.2	31.0	49.0	61.0	25.0	10.0	21.0	30.0	17.0
13 Diphenylhydantoin 300 mg	13	F	52	14.8	30.1	36.8	18.7	21.0	53.0	55.0	31.0	7.5	11.0	12.0	10.0
14 Diphenylhydantoin 300 mg Diazepam 10 mg	14	M	40	14.5	24.0	27.5	12.2	33.0	47.0	48.0	35.0	8.0	15.0	19.0	10.5
15 —	15	F	52	15.6	47.2	41.6	—	20.0	83.0	47.0	—	10.0	17.0	15.5	—
16 —	16	M	32	13.9	23.9	27.4	20.6	27.0	50.0	39.0	41.0	13.5	25.0	19.0	17.5
17 Carbamazepine 600 mg Diphenylhydantoin 300 mg	17	M	16	11.2	20.4	41.4	26.1	14.0	33.0	34.0	24.0	3.0	10.5	10.5	7.0
18 —	18	M	42	15.5	11.0	61.4	4.0	35.0	67.0	28.0	32.0	10.0	19.5	17.5	9.5
19 Carbamazepine 800 mg	19	F	39	19.4	49.8	53.1	44.9	10.0	29.0	25.0	17.0	1.8	2.0	4.0	4.9

Table 15

Excretion of Porter Silber 17 OHCS Ketogenic 17 OHCS and 17 KS (mg/24 hours) *in two day corticotropin test, i m 60 I U and 20 I U gelatine corticotropin twice daily* Individual values *grouped according to the size of the excretion of ketogenic 17 OHCS on the second ACTH day or on the following day*

Other Treatment Dose/day	No of pat	Sex	Age years	Porter Silber 17 OHCS C	Porter Silber 17 OHCS I	Porter Silber 17 OHCS II	Porter Silber 17 OHCS Fo	Ketogenic 17 OHCS C	Ketogenic 17 OHCS I	Ketogenic 17 OHCS II	Ketogenic 17 OHCS Fo	17 KS C	17 KS I	17 KS II	17 KS Fo
60 I U															
1 —	1	M	35	7.5	14.8	44.2	28.0	31.0	47.0	87.0	53.0	13.0	12.0	19.0	20.5
2 —	2	M	52	20.0	30.1	41.4	9.2	54.0	62.0	83.0	53.0	8.5	12.5	17.0	16.0
3 —	3	M	27	10.2	23.0	21.6	14.8	33.0	68.0	82.0	47.0	14.7	22.5	23.0	15.0
4 —	4	M	42	12.5	30.9	36.9	21.8	19.0	48.0	66.0	54.0	15.0	27.0	20.5	21.0
5 —	5	F	49	11.2	20.9	38.4	5.0	38.0	49.0	58.0	36.0	12.5	15.0	18.0	11.0
6 Diphenylhydantoin 200 mg, Phenobarbital 100 mg	6	F	52	4.9	22.8	29.4	10.8	8.6	4.0	59.0	32.0	2.9	13.5	18.5	11.0
7 —	7	F	53	4.8	19.2	39.0	15.0	20.0	29.0	49.0	36.0	12.0	15.5	27.5	14.5
8 —	8	M	57	12.6	12.0	18.5	4.0	43.0	27.0	37.0	21.0	12.0	12.5	14.5	17.0
9 Diphenylhydantoin 300 mg	9	M	64	14.8	25.0	30.9	7.0	30.0	33.0	30.0	17.0	7.0	7.8	9.5	5.6
20 I U															
1 —	1	M	48	10.0	22.0	37.1	5.1	38.0	60.0	115.0	58.0	9.5	18.0	27.5	15.5
2 —	2	M	43	22.0	35.7	31.9	35.4	55.0	73.0	88.0	78.0	11.5	23.0	20.0	16.5
3 —	3	M	42	11.0	28.2	31.0	23.5	32.0	49.0	17.0	73.0	11.0	13.5	18.5	16.0
4 —	4	F	35	20.5	33.5	42.5	23.1	34.0	57.0	69.0	46.0	9.5	12.0	10.5	8.0
5 —	5	M	57	9.2	18.9	18.9	8.1	32.0	65.0	63.0	50.0	7.5	8.4	8.2	4.8
6 —	6	F	40	12.3	34.0	43.7	9.5	14.0	58.0	58.5	27.0	3.8	6.4	10.0	2.7
7 —	7	M	49	11.0	20.0	11.5	17.2	20.0	38.0	52.0	35.0	17.5	20.5	28.5	22.0
8 Chlordiazepoxide 15 mg	8	M	35	8.2	24.0	25.2	12.0	42.0	55.0	27.0	45.0	8.5	10.5	13.0	10.0
9 Diphenylhydantoin 300 mg	9	F	67	8.4	19.1	29.4	44.1	15.0	29.0	43.0	38.0	10.0	15.3	19.0	27.0
10 —	10	M	67	6.0	23.6	18.0	9.0	32.0	43.0	42.0	33.0	5.7	5.8	4.9	5.3
11 —	11	M	48	9.6	8.9	8.4	6.0	29.0	28.0	22.0	41.0	14.5	9.0	8.0	9.5
12 Diphenylhydantoin 300 mg	12	M	52	8.0	13.2	13.3	3.0	36.0	31.0	75.0	16.0	8.0	6.0	7.0	3.5
13 —	13	F	62	8.4	12.0	20.3	10.8	12.0	10.0	26.0	11.5	4.3	5.5	5.3	5.1

C=Control, I=ACTH, II=ACTH, Fo=Following day
F=Female, M=Male

DISCUSSION

The results of the present investigation show that the use of the right doses of the long acting corticotropin preparations in the two day intramuscular corticotropin test gives a good and, from the point of view of clinical diagnosis, useful evaluation of the nearly maximal functional capacity of the adrenal cortex. The two day intramuscular corticotropin test is more recommendable than the one day test, as in earlier corticotropin tests in various clinical materials (e.g. PEKKARINEN & KALLIOMAKI 1958, PEKKARINEN *et al.* 1959, BIRKE *et al.* 1960, PEKKARINEN & SONCK 1962). On the second corticotropin day the mean increase of steroid excretion is *always much higher than on the first corticotropin day, because the adrenocortical* cell activities are already stimulated by corticotropin on the first day. The increase of excretion of Porter-Silber 17-OHCS is nearly twice or more higher on the second corticotropin day than that on the first corticotropin day, while the mean excretion of ketogenic 17-OHCS is nearly 1.5 to 2 times higher and that of 17-KS nearly 1.5 to 3 times higher than on the first corticotropin day. The mean responses of excretions of Porter-Silber 17-OHCS and ketogenic 17-OHCS above the basal excretion are often each of the same size and normally 3—5 times larger than the mean response of 17-KS although the basal excretion of ketogenic 17-OHCS is higher than Porter-Silber 17-OHCS. When long acting corticotropin preparations are purified, we have observed that older reserves (before 1958) have been smaller than newer reserves.

There are clear differences in the adrenocortical potencies of various long acting corticotropin preparations and also large individual variations with the same dose and the same type of corticotropin preparation in the intramuscular test according to our investigations. It confirms the results obtained by studying the increase of the 17-OHCS concentrations in the plasma (HANGÅRD *et al.* 1960, HEDNER 1963). A unification of long acting corticotropin preparations is needed. The maximal adrenocortical response of excretion in two day intramuscular depot corticotropin test of our studies was obtained with only 40 I.U. of polyphloretin phosphate corticotropin twice daily and 60 I.U. no longer raised that often already maximal response. Obviously it is difficult to raise the maximal adrenocortical excretion even with larger corticotropin doses. A similar nearly maximal response of excretion was caused by 60 I.U. of carboxymethyl cellulose corticotropin *and 120 I.U. of zinc hydroxide* corticotropin as with 40 or 60 I.U. of polyphloretin phosphate corticotropin. With gelatine

corticotropin however the corresponding mean responses of excretions both to 120 I U and 60 I U were somewhat although statistically significantly smaller only for the excretion of Porter Silber 17 OHCS than with 120 I U of zinc hydroxide corticotropin

By means of the rise of plasma 17 OHCS content the potentiating and prolonging effect of polyphloretin phosphate corticotropin is stronger than that obtained with gelatine zinc hydroxide or carboxymethyl cellulose long acting corticotropin preparations in order of their intensities of adrenocortical responses (HEDNER 1963) The higher response is also confirmed by our results of excretion of Porter Silber 17 OHCS ketogenic 17 OHCS and 17 KS for polyphloretin phosphate corticotropin in the intramuscular two day corticotropin tests Also the response with carboxymethyl cellulose corticotropin (60 I U) is high in the intramuscular test The zinc hydroxide preparations would be in this respect more effective than gelatine corticotropin (GELLER *et al* 1957 SIEGEL *et al* 1958) According to our findings the rise of the steroid excretion obtained with zinc hydroxide corticotropin is more prolonged still continuing on the day following the two corticotropin days in contrast with the rises caused by any other intramuscular long acting corticotropin preparations *Therefore, in* higher doses the zinc hydroxide corticotropin preparations can cause more accumulation in steroid excretions and smaller doses can maintain the same level in the chronic treatment than higher doses in acute *experiments*

The results obtained on potencies with 20 I U corticotropin on the *other hand* deviated from the maximal adrenocortical reserves obtained with higher doses 40—120 I U With 20 I U of zinc hydroxide and carboxymethyl cellulose corticotropins a somewhat greater adrenocortical responses were obtained than with polyphloretin *phosphate* and gelatine corticotropins while higher doses 40—60 I U of polyphloretin *phosphate* preparations were more effective than other long acting *preparations* It is difficult to compare in clinical diagnosis the *responses* obtained by *different* types of long acting intramuscular corticotropin preparations in the intramuscular *tests in* different dose levels In addition the *clinical* use of new, more purified corticotropin *preparations* with new and higher amounts of units in the *intramuscular* test brought about *uncertainty* in the beginning of their clinical use *in man* Our *investigation showed* that the new type of zinc hydroxide corticotropin *in a dose* almost three times higher, 120 I U causes a nearly equal although *somewhat* higher and good mean response than the 40 I U older type of zinc hydroxide *corticotropin* in the intramuscular two-day corticotropin test *which* preparations we have previously used in our *adrenocortical* function *tests* Because the response with 120 I U of new type of zinc hydroxide corticotropin is higher, although *not* statistically *significantly* higher than with 40 I U *of* old type of zinc

hydroxide corticotropin, we believe, that the new type of zinc hydroxide corticotropin causes a good adrenocortical response with almost 90 or 75 I U twice daily during 2 day's test Differences in the mean steroid excretions between these two doses of old (40 I U) and new (120 I U) corticotropin preparations, however, are not significant

The excretion of the urinary steroids in the two day corticotropin test does not always increase in a clear linear proportion of logarithmic scale of corticotropin dose, e g differences of 40 and 60 I U of polyphloretin phosphate corticotropin and of 20 and 40 I U of zinc hydroxide corticotropin Differences in the steroid excretion are also often small in the logarithmic scale of 2 Metabolic and absorption factors cause a great biological variability in the size of adrenocortical reserves, when they are compared at different dose levels of same corticotropin preparations with *the same dose Many factors influence the size of adrenocortical reserves* and the steroid excretion into the urine the patients' age, general condition and weight, the absorption at the site of injection distribution in the organism, the blood flow, the functional state of the adrenal cortex, the production and secretion rates of corticosteroids from the adrenals, their metabolic conversion and conjugation rates in the liver and other tissues and their renal clearance rate It is important to understand the influence of all these factors in the adrenocortical function and its clinical evaluation and treat*ment of patient Different types of prolonging agents are greatly modifying* the effects of corticotropins on the adrenocortical responses to the same amount of corticotropin units in comparison to their potencies in the standardization as water soluble corticotropins without prolonging agents In 4 or 6 point assays of long acting corticotropin preparations doses must increase in the logarithm of 3, in order to be able to differentiate clearly between the adrenocortical responses of different corticotropin doses, as used also e g for water soluble corticotropin in our bioassays based upon *the determination of plasma free 17 OHCS in guinea pigs (PEKKARINEN, 1964, 1965)*

SUMMARY

The adrenocortical responses and their dose relationships to different long acting corticotropin preparations polyphloretin phosphate (I, Reacthin®), carboxymethyl cellulose (II, Acton prolongatum®), zinc hydroxide (III Cortrophine Z®) and gelatine (IV, Depo ACTH®) corticotropins were studied in the two-day intramuscular corticotropin test in man The functional capacity of the adrenal cortex was assessed by the urinary excretion of Porter Silber 17 OHCS, ketogenic 17 OHCS and 17 KS

According to the size of the maximum adrenocortical responses to great dose of corticotropin (60 or 120 I U) on the second corticotropin day, the order of the intensity of the responses was the following polyphloretin phosphate carboxymethyl cellulose zinc hydroxide and gelatine corticotropin

(1) On the second corticotropin day 40 I U of polyphloretin phosphate corticotropin caused the maximal mean response of excretion per 24 hrs above the mean basal excretion +49 5 mg Porter Silber 17 OHCS, +60 2 mg ketogenic 17 OHCS and 11 0 mg 17 KS, 60 I U caused nearly similar responses

(2) The mean response to 60 I U of carboxymethyl cellulose corticotropin (+49 1 mg +46 6 mg and +11 9 mg respectively) was nearly of the same size as that to 40 and 60 I U of polyphloretin phosphate corticotropin

(3) The mean response with 120 I U of the new type of zinc hydroxide corticotropin (+52 2 mg +51 7 mg and +15 0 mg respectively) corresponded to that with 40 or 60 I U of polyphloretin phosphate corticotropin and that with 60 I U of carboxymethyl cellulose corticotropin showing a higher mean response only for 17 KS It was not significantly ($P < 0\ 05$) greater than that with 40 I U of the old type of zinc hydroxide corticotropin (+43 7 mg +44 5 mg and +10 5 mg respectively) The response with zinc hydroxide corticotropin continues appreciably longer than that with polyphloretin phosphate carboxymethyl cellulose and gelatine corticotropin on the day following the 2 corticotropin days indicating that zinc hydroxide corticotropin gives a longer adrenocortical [illegible] was smaller than with 120 I U of zinc hydroxide corticotropin Only the mean excretion of Porter Silber 17 OHCS with 120 I U of gelatine corticotropin was on the second

hydroxide corticotropin we believe that the new type of zinc hydroxide corticotropin causes a good adrenocortical response with almost 90 or 75 I U twice daily during 2 days test Differences in the mean steroid excretions between these two doses of old (40 I U) and new (120 I U) corticotropin preparations however are not significant

The excretion of the urinary steroids in the two day corticotropin test does not always increase in a clear linear proportion of logarithmic scale of corticotropin dose e g differences of 40 and 60 I U of polyphloretin phosphate corticotropin and of 20 and 40 I U of zinc hydroxide corticotropin Differences in the steroid excretion are also often small in the logarithmic scale of 2 Metabolic and absorption factors cause a great biological variability in the size of adrenocortical reserves when they are compared at different dose levels of same corticotropin preparations with the same dose Many factors influence the size of adrenocortical reserves and the steroid excretion into the urine the patients age general condition and weight the absorption at the site of injection distribution in the organism the blood flow the functional state of the adrenal cortex the production and secretion rates of corticosteroids from the adrenals their metabolic conversion and conjugation rates in the liver and other tissues and their renal clearance rate It is important to understand the influence of all these factors in the adrenocortical function and its clinical evaluation and treatment of patient Different types of prolonging agents are greatly modifying the effects of corticotropins on the adrenocortical responses to the same amount of corticotropin units in comparison to their potencies in the standardization as water soluble corticotropins without prolonging agents In 4 or 6 point assays of long acting corticotropin preparations doses must increase in the logarithm of 3 in order to be able to differentiate clearly between the adrenocortical responses of different corticotropin doses as used also e g for water soluble corticotropin in our bioassays based upon the determination of plasma free 17 OHCS in guinea pigs (PEKKARINEN 1964 1965)

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corticotropin day, significantly smaller than with 120 I U of zinc hydroxide corticotropin ($P < 0\ 05$) The response with 60 I U of gelatine corticotropin was only about a half of the corresponding responses of 40 or 60 I U of polyphloretin phosphate corticotropin and 60 I U of carboxymethyl cellulose corticotropin

The adrenocortical responses caused by 20 I U with zinc hydroxide and carboxymethyl cellulose corticotropin were somewhat higher than with polyphloretin phosphate and gelatine corticotropin on the second corticotropin day However the excretion of Porter Silber 17 OHCS was significantly higher only between zinc hydroxide corticotropin > polyphloretin phosphate corticotropin ($P < 0\ 01$) zinc hydroxide corticotropin > gelatine corticotropin ($P < 0\ 01$) and that of 17 KS between zinc hydroxide corticotropin > polyphloretin phosphate corticotropin ($P < 0\ 01$) and carboxymethyl cellulose corticotropin > polyphloretin phosphate corticotropin ($P < 0\ 01$)

Our results show that there are great differences in the clinical activities of corticotropins with various long acting substances A clinical unification of the long acting corticotropin preparations is needed The present pharmacopoea methods estimate only the activities of water soluble or gelatine corticotropin preparations The excretion of urinary steroids in the two day corticotropin test does not always increase in clear linear proportion of logarithmic scale (e g difference between 20 and 40 I U of zinc hydroxide corticotropin and between 40 and 60 I U of polyphloretin phosphate corticotropin) Metabolic and absorption factors cause a certain variability in the size of the adrenocortical reserves when they are compared at different dose levels of corticotropin preparations which is important clinically to understand in the evaluation of adrenocortical function and in the treatment of patients

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Introduction

By

B Åblad

Ahlquist's classification of adrenergic receptors in one α and one β type (Ahlquist 1948) turned out to be of great value for the subsequent exploration of adrenergic mechanisms However, Ahlquist's concepts did not apparently become more widely accepted until 1958, when Powell & Slater described the pharmacology of dichloroisoprenaline, DCI, the first β adrenergic receptor antagonist without influence on α-adrenergic receptors In subsequent years DCI was frequently used as a tool in studies of adrenergic mechanisms DCI had, besides β-receptor blocking activity, also a pronounced β-receptor stimulating action (Moran & Perkins 1958, Dresel 1960) and it never attained clinical use The chemical structure of DCI gave, however, a starting-point for the development of more selective β-receptor blockers The first clinically used antagonist was pronethalol (Black *et al* 1962) which was later replaced by the considerably more potent and selective agent propranolol (Black *et al* 1964)

One important incitement for the development of β receptor antagonists was the possibility that such agents could be of therapeutic value in certain cardiovascular diseases An excessive cardiac sympathetic drive could be expected to provoke angina pectoris attacks and also to elicit cardiac arrhythmias A β-receptor antagonist might, therefore, be a valuable adjunct in the therapy of these conditions In many cases of heart failure, on the other hand, the cardiac sympathetic tone could be expected to be of importance for the maintenance of the function of the cardiac pump This condition might, therefore, be aggravated by β-receptor antagonists These hypotheses seem to have been verified by clinical experience with β-receptor blocking agents, judging by the studies reported in recent years (review by Epstein & Braunwald 1966)

At Hässle laboratories a research program, aiming at developing a β-receptor antagonist, was started in 1961 The direction of this work was determined by the above outlined hypothetical considerations regarding the possible advantages and drawbacks of β-receptor blockade in the clinic The work was directed at developing a potent β-receptor antagonist possessing a highly selective action, which should, however, include a moderate β-receptor stimulation The β-receptor blocking component should inhibit the cardiac effects

intravenous (VIII) and oral administration (VII) The results indicate that when administered intravenously, the two agents are equipotent antagonists When administered orally, H 56/28 had to be given in doses twice as high as propranolol to produce equal β-receptor blockade Equipotent doses of H 56/28 and propranolol had the same time effect relationship Maximal blockade was seen 10 minutes after intravenous administration and 1–1½ hours after oral administration The approximate ratio between intravenous and oral doses of H 56/28 producing equal maximal blockade was 1 10

Studies of the two optical isomers of H 56/28 (II, IV, V, VII) indicate that the β-receptor blocking and stimulating actions of the racemate are mainly exerted by the laevo form, while the dextro form has very little activity

The dextro and laevo isomers were, however, approximately equally active in clearing ouabain induced ventricular tachycardia in dogs (III) The dextro isomers of pronethalol and propranolol, which have very little β-receptor blocking activity, have also been found to be equipotent to the corresponding racemates as regards clearing effect on ouabain induced ventricular tachycardia in dogs (LUCCHESI 1964, Lucchesi *et al* 1967) The effect of the agents on this experimental arrhythmia could obviously not be due to β receptor blockade, but was ascribed to a quinidine like action (cf SEKIYA & VAUGHAN WILLIAMS 1963, MORALES-AGUILERA & VAUGHAN WILLIAMS 1965) These animal studies have raised the question whether also the clinical antiarrhythmic effects of the racemic forms of these agents could be due to a quinidine like action This would *a priori* appear less likely, since the laevo and racemic forms of the agents cleared experimental ouabain arrhythmia only in doses far above those producing marked β-receptor blockade Studies of the effects of the racemic and dextro forms of H 56/28 in patients with various types of arrhythmia (LINKO *et al* 1967 and personal communication, see III) also indicate that the clinical antiarrhythmic action of racemic H 56/28 is only due to β-receptor blockade

The clinical effects of H 56/28 in various diseases are now under investigation Hitherto reported studies indicate that H 56/28 is an effective and well tolerated therapeutic agent in angina pectoris (BJÖRNTORP 1967) and cardiac arrhythmias (LINKO *et al* 1967) The clinical significance of the β-receptor stimulating action of the drug is under evaluation

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of excessive adrenergic stimuli The *β*-receptor stimulating component should keep the adrenergic receptors in the heart moderately activated, thereby reducing the risk of aggravating cardiac failure

Out of more than one hundred substances synthesized and tested, the benzene derivative H 56/28[1], 1-(o-allylphenoxy)-3-isopropylamino-2-propanol hydrochloride (BRÄNDSTRÖM *et al* 1966), was finally selected for clinical trials

The following seven studies are concerned with the pharmacology of H 56/28 in animals and man Cardiovascular investigations in animals (II)[2] indicate that the predominant action of H 56/28 is *β*-adrenergic receptor blockade The compound was equipotent to propranolol H 56/28 was further found to exert a weak direct *β*-receptor stimulating action Propranolol, which was devoid of this property, consistently reduced basal cardiac output, rate and contractile force because of inhibition of endogenous cardiac sympathetic drive H 56/28, on the other hand, induced only slight changes of these hemodynamic parameters, probably because its inhibitory effect on endogenous cardiac sympathetic tone was counterbalanced by its *β* receptor stimulating action on the heart

A direct parallel to these animal observations was found in a hemodynamic investigation on healthy human subjects (VI) In this study propranolol (10 mg i v) and H 56/28 (10 mg i v) were found to produce equal inhibition of the cardiovascular responses to isoprenaline Resting cardiac output was consistently reduced after propranolol, by 22% on the average After H 56/28, however, cardiac output was not significantly changed

A further comparison was made of the effects of H 56/28 and propranolol on the blood flow in the forearm (V) The results of this study, as well as those obtained in peripheral circulation studies in animals (II), indicate that the peripheral vascular effects of H 56/28 are also determined by a *β* receptor blocking action combined with a weak *β* receptor stimulating activity

The lipolytic action of catecholamines is inhibited by *β*-receptor antagonists (EPSTEIN & BRAUNWALD 1966) H 56/28 was found to antagonize noradrenaline-induced lipolysis in isolated adipose tissue (IV) The agent also blocked the increase of serum free fatty acid concentration produced by noradrenaline infusion in dogs It was further found that the increase of serum free fatty acid concentration, occuring during and after exercise in humans, was partially inhibited by H 56/28 treatment This effect was probably due to adrenergic blockade of lipolysis

The potency and time-effect relationship of H 56/28 and propranolol, as regards *β* receptor blockade, have been studied in human subjects, both after

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[1] Aptin®, AB Hässle, Göteborg Sweden

[2] The Roman numerals II–VIII refer to the present investigations as indicated in the list of contents page 3

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From AB Hässle Department of Pharmacology Göteborg Sweden

Pharmacological Properties of H 56/28—a ß-Adrenergic Receptor Antagonist

By

B ÅBLAD, M BROGÅRD and L EK

The observations that dichloroisoprenaline (DCI) could block β adrenergic receptors (POWELL & SLATER 1958, MORAN & PERKINS 1958) initiated a search for other β-receptor antagonists with isoprenaline related structure In this laboratory for example, a series of phenylethanolamines and phenylpropanolamines was synthesized and several compounds were found to give β adrenergic receptor blockade (CORRODI *et al* 1963) However, in a subsequently synthesized series of phenoxypropanolamines (BRANDSTRÖM *et al* 1966) considerably more potent and selective β receptor antagonists were found One of these compounds 1 (o-allylphenoxy) 3 isopropylamino 2-propanol hydrochloride, H 56/28[1] (fig 1), is now undergoing clinical evaluation in the therapy of various cardiovascular disorders

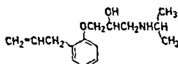


Fig 1 Structure of H 56 28

In the present study some pharmacological properties of H 56/28 will be described and compared to those of another β-adrenergic receptor blocking agent the naphtol derivative propranolol (BLACK *et al* 1964) The investigation deals primarily with the cardiovascular effects of these compounds in various animal *in vivo* and *in vitro* preparations

[1] Aptin®

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Powell, C E & I H Slater Blocking of inhibitory adrenergic receptors of dichloro analogue of isoproterenol *J Pharmacol Exp Ther* 1958, 122, 480–488

Sekiya A & E M Vaughan Williams Effect of pronethalol, dichloroisoprenaline and disopyramide on toxicity to heart of ouabain and anaesthetics *Brit J Pharmacol* 1963, 21, 462–472

2 *Anaesthetized dogs*

Mongrel dogs weighing between 9 and 18 kg were anaesthetized with morphine hydrochloride (2 5 mg/kg i m), diallymal sodium (35 mg/kg i v) and urethane (300 mg/kg i v) The chest was opened through the fourth left intercostal space and the lungs were artificially ventilated The aortic blood flow was determined using a Biotronics electromagnetic flowmeter, the probe being placed around the base of the aorta The femoral arterial mean blood pressure and the left atrial mean blood pressure were measured using Statham transducers ECG (extremity lead II) was also registered Recordings were made on an Offner Dynograph Peripheral vascular resistance was estimated and expressed in "units" obtained by dividing the mean arterial blood pressure (in mm Hg) by the aortic blood flow (in l/minute)

C *Test drugs*

The following drugs were used racemic 1-(o-allylphenoxy)-3 isopropylamino-2 propanol hydrochloride (in the following called H 56/28), the laevo isomer of H 56/28 as *bitartrate*, the dextro isomer of H 56/28 as hydrochloride, propranolol hydrochloride, pronethalol hydrochloride, laevo isoprenaline bitartrate, laevo noradrenaline bitartrate, pilocarpine hydrochloride, calcium chloride, ouabain, theophyllamine, reserpine, tyramine hydrochloride, atropine sulphate Doses of the laevo isomer of H 56/28 refer to the *hydrochloride; all* other drug forms were as listed above

The results are given as means ± s e m.

Results

A *In vitro experiments*

1 *Effect of H 56/28 on papillary muscle*

a) Influence on the inotropic response to isoprenaline.

Cumulative dose-response curves for isoprenaline were determined on an isolated, electrically driven papillary muscle before and after treatment with H 56/28

Geometrically increasing doses of isoprenaline were added to the bath, each dose producing its full effect before addition of the next higher dose, until the maximum response was ob- [illegible] absence of H 56/28 In each experiment the influence of H 56 28 on the isoprenaline ED 50 (i e the dose of isoprenaline producing a half maximal response) was calculated.

Four experiments were performed In two control experiments where the muscles were not exposed to H 56/28 repeated determinations of cumulative dose response curves for isoprenaline gave reproducible results.

Fig 2 shows the mean results obtained Treatment with H 56/28 caused a parallel shift to the right of the dose-response curve for isoprenaline without reducing its maximal effect The two concentrations of H 56/28 (0 005 and

Methods

A *In vitro experiments*

1. *Papillary muscle*

Papillary muscles from the right ventricle of rabbits weighing about 2 kg were mounted in a jacketed organ bath containing Krebs' solution at 37°C (KREBS & HENSELEIT 1932) The solution was aerated by a mixture of 93 5% oxygen and 6 5% carbon dioxide Platinum surface electrodes were attached and the muscle was electrically driven throughout the experiment by pulses of 5 msec. duration and 3–5 volts strength at a frequency of one impulse per second Isometric contractions were recorded by means of a force displacement transducer and an Offner Dynograph The resting tension was individually adjusted for each muscle varying between 0 2 and 1 g and the contractile force prior to the addition of drugs was 0 5–1 5 g

2 *Tracheal smooth muscle*

The trachea from guinea pigs weighing 300–500 g was removed, cut into rings of equal width and tied together to form a chain of 10 rings as described by FOSTER (1960) The chain was transferred to an organ bath containing aerated Krebs' solution at 37°C The Krebs' solution contained pilocarpine hydrochloride (1 mg/l) to induce tension in the muscle The chain was attached to a force displacement transducer, the rings were opened by cutting through the cartilage and the chain was thereafter put under a tension of 1 6 g Isometric recordings were made on an Offner Dynograph The tissue was left in the bath for about one hour before the addition of drugs

B *In vivo experiments*

1 *Anaesthetized cats*

Cats weighing between 1 8 and 3 4 kg were anaesthetized with chloralose (60 mg/kg i v) or pentobarbital sodium (30 mg/kg i p) A tracheal cannula was inserted and in experiments where thoracotomy or vagotomy was to be performed, artificial respiration was given and adjusted to a level that just suppressed the spontaneous respiration of the animal Arterial blood pressure was recorded from a cannula in one carotid artery with a Statham transducer Heart rate was calculated from the blood pressure pulses or recorded by a cardiotachometer In some experiments the chest was opened through the sternum and cardiac contractile force was measured by means of a small Brodie Walton strain gauge arch sutured to the right ventricle near its base The initial diastolic tension was adjusted to about 20 g All recordings were made on an Offner Dynograph

Drugs were administered intravenously by injection or by infusion using a constant flow infusion pump In some experiments peripheral vagal or cardiac sympathetic nerves were stimulated using bipolar electrodes and a Grass Model S 4 stimulator

In the results cardiac contractile force values are expressed in "units" derived in the following manner In each experiment the absolute value of the initial cardiac contractile force recorded immediately before the administration of the first dose of an antagonist was taken as 100 "units" The absolute levels for the contractile force subsequently recorded in the course of the experiment were then related to this value and recalculated as "units"

2 *Anaesthetized dogs*

Mongrel dogs weighing between 9 and 18 kg were anaesthetized with morphine hydrochloride (2.5 mg/kg i m.), diallymal sodium (35 mg/kg i.v.) and urethane (300 mg/kg i v.) The chest was opened through the fourth left intercostal space and the lungs were artificially ventilated. The aortic blood flow was determined using a Biotronics electromagnetic flowmeter, the probe being placed around the base of the aorta The femoral arterial mean blood pressure and the left atrial mean blood pressure were measured using Statham transducers ECG (extremity lead II) was also registered Recordings were made on an Offner Dynograph Peripheral vascular resistance was estimated and expressed in "units" obtained by dividing the mean arterial blood pressure (in mm Hg) by the aortic blood flow (in l/minute)

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Geometrically increasing doses of isoprenaline were added to the bath, each dose producing its full effect before addition of the next higher dose, *until* the maximum response was obtained Subsequently the perfusing Krebs' solution was changed Then H 56/28 [illegible]
to the bath in a [illegible]
curve for isopren[illegible]
made 45 minutes [illegible]
Results were [illegible]
produced by isop[illegible] [illegible] each experiment the influence of
H 56/28 on the isoprenaline ED 50 (i e the dose of isopren[illegible]
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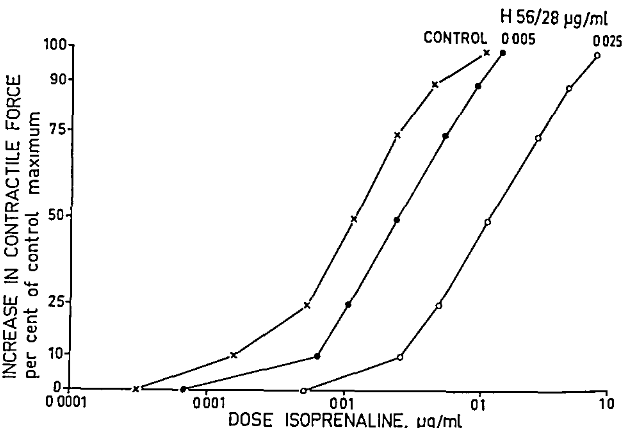


Fig 2 Electrically driven papillary muscle suspended in Krebs' solution Contractile force recorded Cumulative isoprenaline dose response curves before and after addition of H 56/28 in concentrations *of 0 005 and 0 025 µg/ml Mean results of four experiments Curves plotted* on the basis of values obtained by estimating the average concentration giving a certain response (cf ARIENS *et al* 1964, pp 144–146)

0 025 *µg/ml) increased the isoprenaline ED 50 by 0 3±0 12 log units and* 1 0±0 14 log units respectively

b) Influence of H 56/28 on the inotropic response to calcium chloride, ouabain or theophyllamine

In order to investigate the selectivity of the action of H 56/28 a study was made of its interference with the positive inotropic effect of calcium chloride, ouabain or theophyllamine on the isolated, electrically driven papillary muscle preparation

In this investigation two muscles, suspended in separate baths, were studied simultaneously The contractile force of the muscles was increased by calcium chloride (0 4-0 8 mg/ml) which was added to the baths every 45 minutes and remained there until its peak effect had been reached When stable, submaximal control responses to calcium chloride had been obtained, one muscle was exposed successively to increasing doses of H 56/28 (0 025 0 25 and 2 5 µg/ml) while the other muscle received physiological saline Forty five minutes

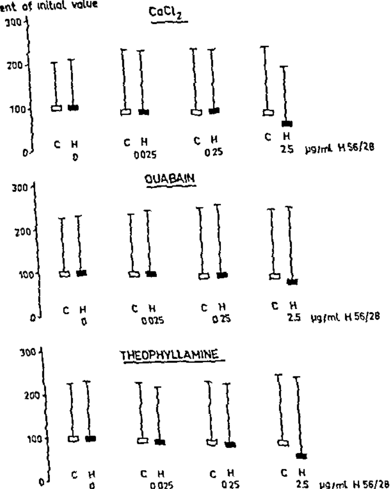


Fig. 3 Electrically driven papillary muscles suspended in Krebs' solution. Inotropic response to calcium chloride, ouabain and theophyllamine before and after treatment with H 56/28 in concentrations of 0 025, 0 25 and 2 5 µg/ml Open rectangles basal contractile force in control muscles Filled rectangles basal contractile force in H 56/28 treated muscles Thin horisontal bars maximal recorded contractile force after addition of submaximal dose of stimulant in control (C), and H 56/28 treated (H) muscles Each set of results is the mean of five experiments

after each addition of H 56/28 (or saline), the effect of calcium chloride was redetermined. The influence of H 56/28 on the positive inotropic effect of ouabain (1 8 mg/ml) or theophyllamine (0.5-0 75 mg/ml) was studied in the same manner as with calcium chloride Five experiments were performed with each cardiac stimulant.

In evaluating the results the basal contractile force recorded previous to the last control response to stimulant was taken as 100% The subsequently recorded values for the contractile force were then related to this value and recalculated as per cent

Fig 3 shows the mean results obtained H 56/28 did not inhibit the positive inotropic effects of calcium chloride, ouabain and theophyllamine even after a dose of 2 5 µg/ml, while a submaximal positive inotropic response to isoprenaline was previously found to be practically abolished by H 56/28 in a dose of 0 025 µg/ml (fig 2) The basal contractile force was not significantly altered by H 56/28 in doses of 0 025 and 0 25 µg/ml, but consistently depressed by the dose of 2 5 µg/ml (fig 3) In the 15 muscles treated with 2 5 µg/ml H 56/28 the basal contractile force was 24±5 3% lower than that simultaneously recorded in the untreated control muscles

2 *Quantitative comparison of the effects of H 56/28, propranolol and pronethalol on responses to isoprenaline*

a) Papillary muscle

A comparison was made of the antagonistic activity of H 56/28, propranolol and pronethalol on the positive inotropic response to isoprenaline in papillary muscle

Isoprenaline, in a dose that produced a submaximal positive inotropic response (0 02 µg/ml) was added to the bath every 15 minutes and remained there until it exerted its peak effect before changing the bath fluid When reproducible control responses had been obtained, an antagonist was added Each dose of the test antagonist was in contact with the muscle until no further blockade of the isoprenaline response was observed On the average, a 'steady state blockade' was obtained after 45 minutes On each muscle 3–4 increasing doses of one antagonist were tested For each experiment a per cent inhibition log dose curve was drawn and the dose producing 50% inhibition of the control response (ED 50) was calculated Each drug was tested in 5–8 experiments

The results (table 1) indicate that H 56/28 had approximately the same activity as propranolol as regards blockade of the inotropic response to isoprenaline whereas pronethalol was considerably less potent The approximate ratio between equipotent antagonistic doses of H 56/28 and pronethalol was 1 10

b) Tracheal muscle

The effects of the three compounds on isoprenaline-induced relaxations of pilocarpine-treated tracheal chains were studied in the same manner as on papillary muscle The dose of isoprenaline was 0 02 µg/ml Each substance was tested on five tracheal chain preparations

H 56/28, propranolol and pronethalol were found to antagonize the relaxing effect of isoprenaline The antagonistic activity of the three agents showed approximately the same quantitative relationship as in papillary muscle (table 1)

Table 1 Blockade of isoprenaline response to a) papillary muscle b) tracheal muscle Values refer to concentration of antagonist required to produce 50% inhibition of inotropic response to isoprenaline (a) and of relaxation produced by isoprenaline (b) Each value represents the mean result of 5-8 experiments

Compound	a) papillary muscle ED 50±s e m. µg/ml	b) tracheal muscle ED 50±s.e m. µg/ml
H 56/28	0 013±0 0038	0 026±0 0078
Propranolol	0 014±0 0031	0 032±0 0055
Pronethalol	0 122±0 036	0.306±0 114

B *In vivo experiments*

1 *Effects of H 56/28 and propranolol on heart rate and blood pressure in the anaesthetized cat*

The effects of H 56/28 and propranolol on heart rate and mean arterial blood pressure and their influence on the action of isoprenaline were studied in cats anaesthetized with chloralose H 56/28 or propranolol was given intravenously over 20 minutes in a dose of 0 5 mg/kg and the response to intravenously injected isoprenaline (0 25 µg/kg) was recorded before and 5, 25 and 45 minutes after administration Four experiments were performed with each antagonist

The mean results obtained are shown in fig 4 The basal heart rate was not significantly changed by H 56/28 whereas a decrease was observed after propranolol Five minutes after the end of drug infusion the heart rate change in the cats given H 56/28 was −1±4 3 beats/minute while the heart rate in the propranolol treated cats was 23±5 3 beats/minute below the pre-drug level Neither H 56/28 nor propranolol influenced the blood pressure significantly

The isoprenaline induced tachycardia and hypotension were considerably reduced after treatment with H 56/28 The blockade was most pronounced five minutes after the end of the infusion, the chronotropic response being reduced by 71±10 6% and the hypotensive response by 76±6 2% A partial blockade persisted 40 minutes later Propranolol produced a quantitatively similar inhibition of the effects of isoprenaline Five minutes after administration the chronotropic response was decreased by 63±12 2% and the hypotensive response by 79±5 2%

2. *Cardiovascular effects of H 56/28 and propranolol in the anaesthetized cat pretreated with reserpine*

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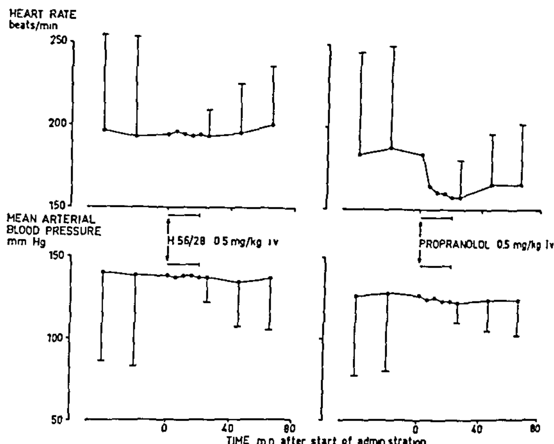


Fig 4 Cats anaesthetised with chloralose Heart rate and arterial blood pressure recorded. Dots basal levels Bars peak responses to i v injected isoprenaline (0 25 μg/kg) H 56/28 and propranolol infused i v for 20 minutes in a dose of 0 5 mg/kg Mean results of four experiments with each antagonist

a) their direct effects on a heart devoid of vegetative nervous control, b) the cardiac effects of combined administration of H 56/28 and propranolol, c) the dose response relationship and d) the time-effect relationship for the inhibitory action of the two agents on the cardiovascular responses to isoprenaline

The studies were performed on cats anaesthetized with pentobarbital 18 hours after treatment with reserpine (5 mg/kg i m) Both adrenal glands were removed and bilateral vagotomy in the neck was performed Heart rate and intraarterial mean blood pressure were recorded *in all experiments* *In two control experiments electrical stimulation* of the right stellate ganglion (stimulation for 30 seconds with 10 impulses/second, impulse strength 10 V and impulse duration 4 msec) or i v injections of tyramine (0 25 mg/kg) produced practically no change of heart rate and blood pressure It was therefore probable that the peripheral catecholamine stores were essentially depleted by the reserpine pretreatment

a) Direct cardiac effects

The direct effects of H 56/28, its two optical isomers and propranolol on cardiac rate and contractile force were *investigated in reserpinized* cats In

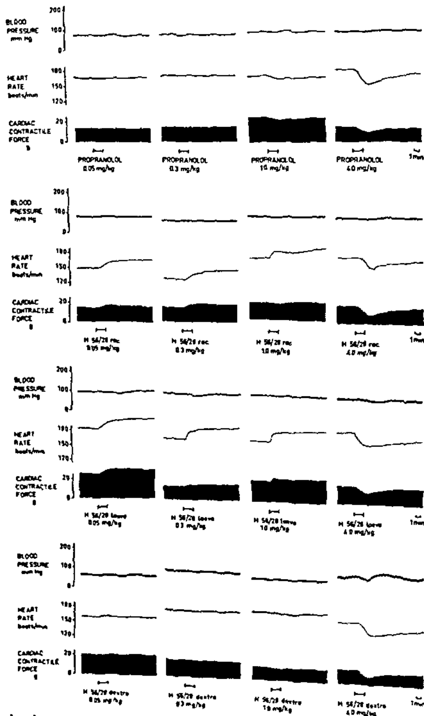


Fig. ... After ... injec... [illegible] ...experiments, in each of which one dose of one agent was tested

each experiment one agent was injected over two minutes in one single dose, varying from 0 05 to 16 mg/kg Thirty experiments were carried out

Fig 5 shows typical results Propranolol produced practically no cardiac effects in doses from 0 05 to 0 3 mg/kg Doses of 0 5 to 1 0 mg/kg as a rule elicited small and transient negative inotropic and chronotropic effects After higher doses of the drug this cardiodepressive effect was more marked Ten minutes after a dose of 4 mg/kg propranolol cardiac contractile force was depressed by about 20% The animals died after a dose of 16 mg/kg with marked cardiac dilatation, slow irregular rhythm and hypotension

Racemic H 56/28, in doses from 0 05 to 1 0 mg/kg elicited moderate positive chronotropic and inotropic effects The heart rate and contractile force started to increase during the injection and reached a "steady state' after about five minutes The heart stimulating effect of the dose of 0 05 mg/kg was quantitatively similar to that of higher doses

After H 56/28 in doses of 0 5 to 1 0 mg/kg a slight transient negative inflection in the heart rate and contractile force recordings was usually seen Higher doses of H 56/28 produced cardiodepressive effects of the same type and magnitude as seen after propranolol administration After H 56/28 in a dose of 16 mg/kg, the animals died with marked cardiodepression and hypotension

The leavo isomer of H 56/28 produced essentially the same cardiac effect pattern as the racemate The dextro isomer of H 56/28 elicited practically no effects in doses from 0 05 to 0 3 mg/kg and after 0 5–1 0 mg/kg only a slight transient cardiodepression was observed Higher doses of this isomer produced marked cardiodepressive effects of the same type and magnitude as propranolol and the racemic and laevo forms of H 56/28 The animals died after a dose of 16 mg/kg of both isomers because of marked cardiodepression

In two further experiments on reserpinized cats the heart was paced at a constant rate of 220 beats/minute by means of electrical stimulation via electrodes sewn to the right atrium H 56/28 (0 05 mg/kg i v) was found to increase cardiac contractility even in these experiments This indicates that the positive inotropic effect of the drug could not solely be due to an increased frequency of cardiac contractions

In order to investigate whether the cardiodepressive effect of the agents was due to cholinergic mechanisms, atropine (1 mg/kg i v) was given ten minutes before the i v injection of racemic H 56/28 or propranolol in a dose of 4 mg/kg to reserpinized cats Two experiments were carried out with each β-receptor antagonist It was found that both H 56/28 and propranolol had the same negative chronotropic and inotropic effects in these experiments as in animals not pretreated with atropine

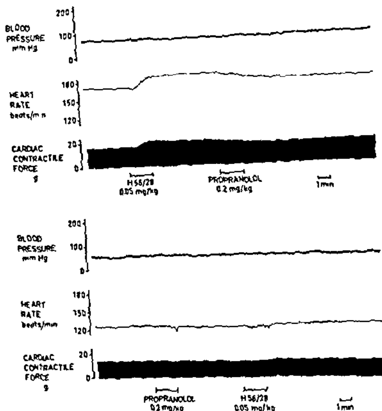


Fig 6 Reserpinized vagotomized, adrenalectomized cats anaesthetized with pentobarbital Arterial blood pressure, heart rate and cardiac contractile force recorded. Influence of propranolol (0 2 mg/kg i.v) on the effects of H 56/28 (0 05 mg/kg i v) Upper recording H 56/28 given six minutes before propranolol Lower recording H 56/28 given six minutes after propranolol

b) Effect of propranolol on the stimulating action of H 56/28

In another study on reserpinized cats the influence of propranolol on the heart stimulating action of H 56/28 was analyzed In four experiments an intravenous injection of 0 05 mg/kg H 56/28 was given and found to increase the heart rate by 21 ± 1 9 beats/minute In four further experiments 0 2 mg/kg propranolol was given intravenously ten minutes before an intravenous injection of 0 05 mg/kg H 56/28 In the latter group of experiments H 56/28 increased the heart rate by 4 ± 0 6 beats/minute Thus the positive chronotropic effect of H 56/28 was inhibited by the propranolol pretreatment

The positive inotropic effect of H 56/28 was also inhibited by propranolol, whether the latter agent was given before or after H 56/28 (fig 6)

each experiment one agent was injected over two minutes in one single dose, varying from 0 05 to 16 mg/kg Thirty experiments were carried out

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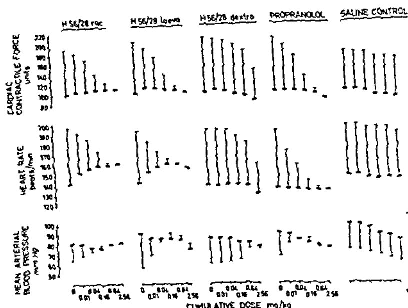


Fig 7 Reserpinized, vagotomized, adrenalectomized cats anaesthetized with pentobarbital Cardiac contractile force, heart rate and mean arterial blood pressure recorded Effects of an i v injected dose of isoprenaline before and after i v administration of H 56 28, its optical isomers or propranolol in increasing doses Thick bars basal levels recorded immediately before injection of isoprenaline Thin bars peak levels recorded after injection of isoprenaline Mean results of five experiments with each agent and four control experiments

The experiments were performed in the following manner An intravenous dose of isoprenaline giving a stable submaximal chronotropic response (usually 0 1 μg/kg) was established (control response) Then H 56/28 or propranolol was given in a dose of 0 1 mg/kg by intravenous infusion over 20 minutes From 10 to 130 minutes after the end of infusion isoprenaline was injected intravenously at 20 minutes intervals in the dose used to obtain the control response.

Table 2. Blockade of positive cardiac chronotropic response to isoprenaline in anaesthetized cat pretreated with reserpine Antagonistic activity of the compounds expressed as the intravenous dose giving 50% inhibition of a submaximal response to isoprenaline Each value represents the mean result of 5 experiments.

Compound	Antagonistic activity ED 50 ± s.e m. mg/kg
H 56/28 racemate	0 04 ± 0 014
H 56/28 laevo	0 02 ± 0 009
H 56 28 dextro	2.0 ± 0 36
Propranolol	0 04 ± 0 012

c) Blockade of cardiovascular responses to isoprenaline dose-response relationship

A comparison was made between H 56/28, its two optical isomers and propranolol as regards direct effects and blockade of isoprenaline-induced responses on heart rate, cardiac contractile force and arterial blood pressure in barbiturized cats pretreated with reserpine

The experiments were carried out in the following manner First a supramaximal cardiac response to isoprenaline was recorded by giving an intravenous injection of 0 5 μg/kg isoprenaline Then the dose of isoprenaline producing cardiac responses that were 70-80% of the maximal responses was established (usually 0 1 μg/kg i v) When stable submaximal control responses to isoprenaline had been obtained, one β receptor antagonist was given in increasing doses at 20 minute intervals, each dose being injected i v over two minutes Ten minutes after each dose of the tested antagonist an intravenous injection of isoprenaline was given in that dose used to establish the control responses The tested antagonist was given in such doses that the cumulative dose increased geometrically 0 01 0 04-0 16-0 64 2 56 mg/kg The dose producing 50% blockade of the chronotropic control response to isoprenaline (ED 50) was estimated in each experiment from curves obtained by plotting percentage inhibition against log cumulative dose

Each antagonist was studied in five experiments Further four control experiments were carried out where physiological saline was given instead of an antagonist

Propranolol and the racemic and laevo forms of H 56/28 markedly reduced the heart stimulating and hypotensive effects of isoprenaline already in a dose of 0 04 mg/kg, and after a dose of 0 64 mg/kg of these agents, the isoprenaline responses were practically abolished (fig 7) The dextro isomer of H 56/28 was much less active Racemic H 56/28 had an antagonistic activity equal to that of propranolol (table 2) The approximate ratio between equipotent antagonistic doses of the laevo and dextro isomers of H 56/28 was 1 100 the laevo isomer being more potent than racemic H 56/28

The basal heart rate and cardiac contractile force increased somewhat after racemic H 56/28 The heart rate reached its peak after 0 16 mg/kg H 56/28 and higher doses did not produce further cardiac stimulation The laevo isomer of H 56 28 increased basal heart rate and cardiac contractile force in the same manner as the racemate The changes of basal cardiac rate and contractile force after propranolol and the dextro isomer of H 56/28 were not significantly different from those obtained in the control experiments

d) Blockade of the chronotropic response to isoprenaline time-effect relationship

A comparative study was made between H 56/28 and propranolol as regards the duration of the blockade of the chronotropic response to isoprenaline Further an attempt was made to compare the time-effect relationships of the heart stimulating and the isoprenaline-antagonistic actions of H 56/28 The study was carried out on barbiturized cats pretreated with reserpine

The basal heart rate increased after H 56/28 whereas a gradual decrease was noted both in the experiments with propranolol and in the control experiments (fig 8) For the evaluation of the positive chronotropic effect of H 56/28, the heart rate changes recorded after this drug were related to those recorded in the control experiments This calculation showed that the positive chronotropic effect of H 56/28 reached its peak at the end of drug administration (difference between heart rates in H 56/28 treated cats and control animals 14±2.6 beats/minute) The effect then gradually decreased, but still 110 minutes later the heart rate in the cats treated with H 56/28 was higher than that in the control animals (difference 8±3 9 beats/minute)

3 *Effects of H 56/28 and propranolol on cardiac rate and contractile force in anaesthetized cats with endogenous cardiac sympathetic tone*

The cardiac effects of H 56/28 and propranolol were further investigated in anaesthetized cats which were not reserpinized and consequently could be expected to have some endogenous cardiac sympathetic tone The studies were directed at evaluating a) the effects of the antagonists on basal heart rate and cardiac contractile force, b) the influence of the two drugs on the cardiac responses to sympathetic nerve stimulation, c) the selectivity of the agents as β receptor antagonists

a) Effects on cardiac rate and contractility

The experiments were performed in cats anaesthetized with pentobarbital Both vagal nerves were cut in the neck Heart rate, cardiac contractile force and mean arterial blood pressure were recorded

H 56 28 or propranolol was administered intravenously in increasing doses 0 05–0 3–0 5–1 0–2 0 mg/kg All doses were slowly injected over five minutes and the interval between two doses was five minutes Twelve experiments were performed

Fig 9 and 10 show typical results with propranolol and H 56/28 respectively Propranolol, in a dose of 0 05 mg/kg, consistently reduced cardiac rate and contractile force The effect was characterized by rapid onset and most often reached its peak at the end of the injection The heart rate and cardiac contractile force then remained practically unchanged during the next five minute period After 0 3 mg/kg propranolol a slight further fall of cardiac rate and contractile force was observed, whereas the following dose of 0 5 mg/kg usually produced no further effect After the dose of 1 0 or 2 0 mg/kg a more transient cardiodepressive effect began to appear The latter effect probably corresponded to the transitory cardiodepression seen in reserpinized cats after 1 mg/kg propranolol (fig 5)

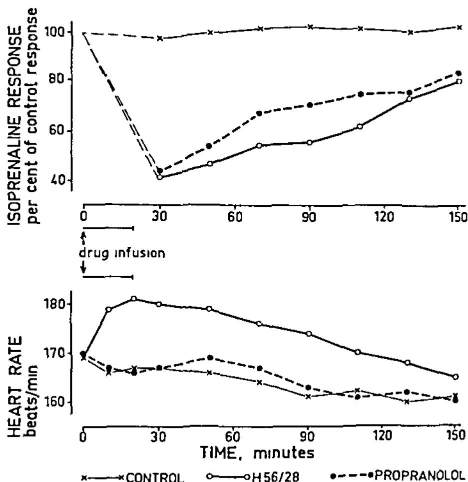


Fig 8 Reserpinized vagotomized, adrenalectomized cats anaesthetized with pentobarbital Heart rate recorded Upper diagram chronotropic responses to repeated injections of isoprenaline Lower diagram basal heart rate levels H 56/28 (0 1 mg/kg) propranolol (0 1 mg kg) or physiological saline (control) infused i v for 20 minutes as indicated Mean results of five experiments with each agent

In each experiment the isoprenaline responses were expressed in per cent of the control response Five experiments were performed with each antagonist In addition five control experiments were made, in which physiological saline was given instead of H 56/28 or propranolol

H 56/28 and propranolol produced approximately equal blockade of the chronotropic response to isoprenaline, both as regards maximal effect and duration of action (fig 8)

The blockade was most marked ten minutes after the end of administration of an antagonist, when the isoprenaline response after H 56/28 was 42 ± 2 2% and after propranolol 44 ± 3 7% of the control response A partial blockade persisted 100 minutes later, when the isoprenaline response after H 56/28 was 74 ± 3 9% and after propranolol 76 ± 8 4% of the control response

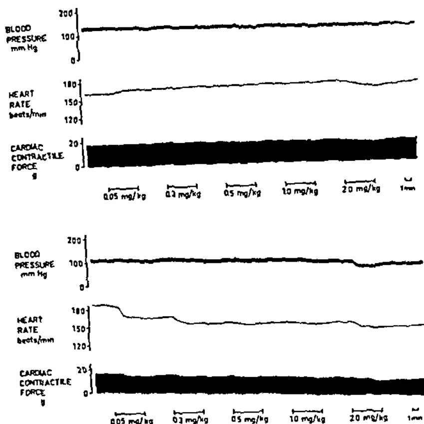


Fig 10 Vagotomized cats anaesthetized with pentobarbital Arterial blood pressure heart rate and cardiac contractile force recorded Effects of increasing i v doses of H 56 28 in two cats Each dose injected over five minutes Interval between each dose five minutes.

b) Effects on cardiac responses to sympathetic nerve stimulation

The effects of increasing doses of H 56/28 or propranolol on the positive chronotropic and inotropic responses to cardiac sympathetic nerve stimulation were studied on barbiturized cats The dose schedule for administration of H 56/28 and propranolol in this study was also used in another investigation, where the effects of the antagonists on basal heart rate in barbiturized cats pretreated with reserpine were analyzed A comparison was made between the effects of the antagonists on basal heart rate in the two studies

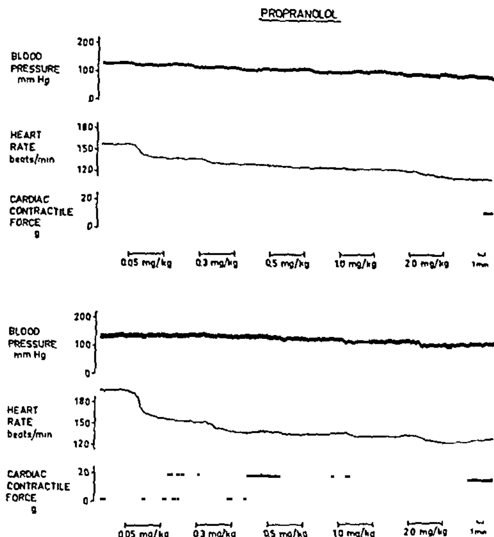


Fig 9 Vagotomized cats anaesthetized with pentobarbital Arterial blood pressure heart rate and cardiac contractile force recorded Effects of increasing i v doses of propranolol in two cats Each dose injected over five minutes Interval between each dose five minutes

H 56/28 usually altered cardiac rate and contractile force very little in the doses of 0 05, 0 3 and 0 5 mg/kg and after the dose of 1 0 or 2 0 mg kg only a transient depression was seen (fig 10, upper tracing) In some animals which had high initial heart rate, H 56/28 produced the same cardiac effect pattern as propranolol with negative chronotropic and inotropic effects after the doses of 0 05 and 0 3 mg/kg and little further change after higher doses except for the transient cardiodepression beginning to appear after the dose of 1 0 or 2 0 mg/kg (fig 10, lower tracing)

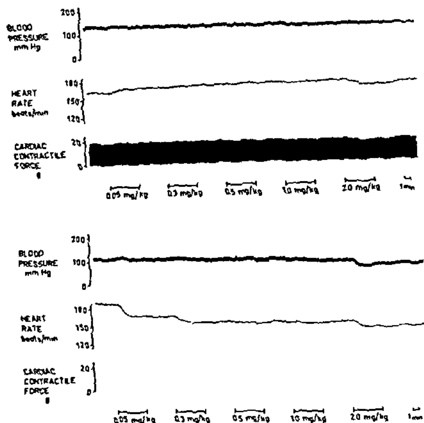


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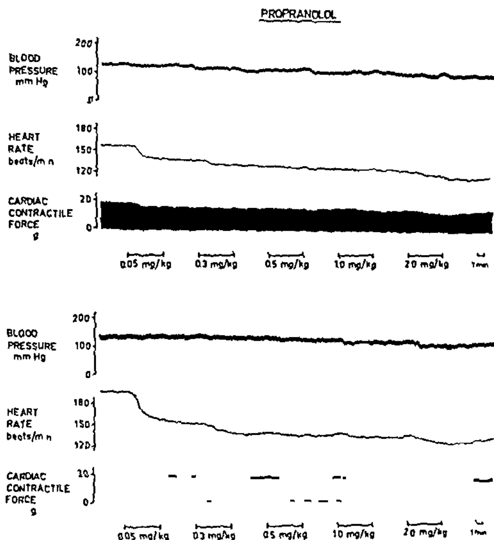


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H 56/28 usually altered cardiac rate and contractile force very little in the doses of 0 05, 0 3 and 0 5 mg/kg, and *after the dose of 1 0 or 2 0 mg kg only* a transient depression was seen (fig 10, upper tracing) In some animals which had high initial heart rate, H 56/28 produced the same cardiac effect pattern as propranolol with negative chronotropic and inotropic effects after the doses of 0 05 and 0 3 mg/kg and little further change after higher doses except for the transient cardiodepression beginning to appear after the dose of 1 0 or 2 0 mg/kg (fig 10, lower tracing)

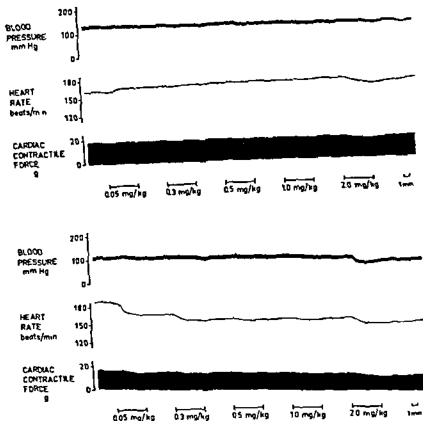


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In the first group of cats the preganglionic fibres to the right stellate ganglion were cut otherwise the sympathetic innervation to the heart was left intact Bilateral vagotomy was carried out in the neck Heart rate and cardiac contractile force were recorded

In these cats the effects of H 56/28 and propranolol on the cardiac responses to sympathetic nerve stimulation were evaluated in the following manner The right stellate ganglion was stimulated for 15 seconds with 10 impulses/sec, 2 msec impulse duration and supra maximal voltage When stabile control responses had been obtained, H 56/28 or propranolol was injected intravenously over two minutes in increasing doses of 0 05-0 1-0 25-0 5-1 0 mg/kg with an interval of 25 minutes between each dose Ten minutes after each dose of antagonist the cardiac response to stellate ganglion stimulation was recorded Four experiments were performed with each antagonist In addition four control experiments were performed, where physiological saline was given instead of antagonist

The other group of cats was studied 18 hours after treatment with reserpine (5 mg/kg i m) Both vagal nerves were cut in the neck and the adrenal glands were removed Heart rate was recorded H 56/28 or propranolol was then administered according to a regimen identical to that in the above mentioned study and heart rate was recorded ten minutes after each dose of antagonist Five experiments were performed with each antagonist Further five control experiments were carried out, where physiological saline was given instead of an antagonist

Fig 11 shows the mean results obtained In the control experiments the cardiac responses to repeated sympathetic nerve stimulation were pratically unchanged H 56/28 and propranolol produced a dose-related inhibition of the positive chronotropic and inotropic effects of sympathetic nerve stimulation After a total dose of 0 4 mg/kg H 56/28 the heart rate increase during sympathetic nerve stimulation was 18±5 0% of the control response and the contractile force increase was 29±4 8% of the control response After a total dose of 0 4 mg/kg propranolol the corresponding values were 30±8 5% for the chronotropic response and 23±10 6% for the inotropic response Thus H 56/28 and propranolol were approximately equipotent inhibitors of the cardiac responses to sympathetic nerve stimulation

H 56/28 and propranolol produced, however, different effects on the basal cardiac rate and contractile force The first dose of H 56/28 (0 05 mg/kg) produced no significant change of heart rate (a mean increase of 4±4 3 beats/minute) or cardiac contractile force (a mean increase of 10±6 1 units) Even after H 56/28 in a total dose of 2 mg/kg the basal heart rate and cardiac contractile force were not reduced below the initial level The first dose of propranolol (0 05 mg/kg) elicited a significant decrease of the basal heart rate (15±4 4 beats/minute) and cardiac contractile force (17±2 2 units) Higher doses of propranolol, up to totally 2 mg/kg, gave only a slight further cardiac depression

In the study on reserpinized cats the first dose of H 56/28 (0 05 mg/kg) increased basal heart rate by 21±1 9 beats/minute Higher doses of H 56/28, up to totally 2 mg/kg, did not give much further change of the basal heart rate The heart rate changes in reserpinized cats given propranolol (0 05 to

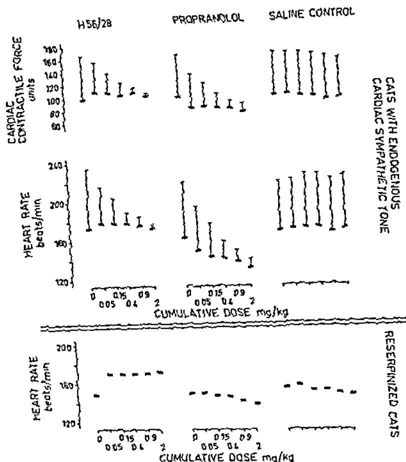


Fig 11 Vagotomized cats anaesthetized with pentobarbital

Above horisontal double line Cardiac effects of sympathetic nerve stim[illegible]

Below horisontal double line Basal *heart rate in reserpinized* cats given H 56/28 or propranolol according to the same dose schedule *as in the above described study* The bars indicate basal heart rate recorded ten minutes after each dose of antagonist *Mean results* of five experiments with each antagonist and five control *experiments*

totally 2 mg/kg) did not differ significantly from those recorded in the control experiments

It is of interest that the difference between the heart rate changes induced by 0 05 mg/kg H 56/28 and propranolol was approximately the same in the cats with largely intact cardiac sympathetic innervation (19±6 1 beats/minute) as in the reserpinized cats (20±2 1 beats/minute)

In the first group of cats the preganglionic fibres to the right stellate ganglion were cut otherwise the sympathetic innervation to the heart was left intact Bilateral vagotomy was carried out in the neck Heart rate and cardiac contractile force were recorded

In these cats the effects of H 56/28 and propranolol on the cardiac responses to sympathetic nerve stimulation were evaluated in the following manner The right stellate ganglion was stimulated for 15 seconds with 10 impulses/sec, 2 msec impulse duration and supramaximal voltage When stabile control responses had been obtained, H 56/28 or propranolol was injected intravenously over two minutes in increasing doses of 0 05-0 1-0 25-0 5-1 0 mg/kg with an interval of 25 minutes between each dose Ten minutes after each dose of antagonist the cardiac response to stellate ganglion stimulation was recorded Four experiments were performed with each antagonist In addition four control experiments were performed, where physiological saline was given instead of antagonist

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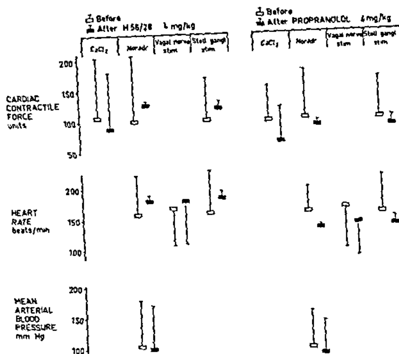


Fig 12 Vagotomized cats anaesthetized with pentobarbital Cardiac contractile force, heart rate and arterial mean blood pressure recorded Effects of calcium chloride (10 mg/kg i v) noradrenaline (0 5 μg kg i v) peripheral vagal and cardiac sympathetic nerve stimulations before and after i v injection of H 56/28 of propranolol in a dose of 4 mg/kg Basal levels recorded before (open rectangles) and after (filled rectangles) administration of antagonist The thin bars indicate peak levels recorded after application of each of the four studied stimuli Mean results of four experiments with each β receptor antagonist

Isoprenaline increased heart rate and aortic flow and reduced peripheral vascular resistance These changes tended to reach a steady state at the end of the infusion of the amine The effects of isoprenaline were reduced markedly and to about the same extent after H 56/28 and propranolol in a dose of 0 1 mg kg and practically abolished after an additional dose of 1 0 mg/kg (table 3)

H 56/28 did not significantly influence cardiac output, heart rate, arterial or left atrial blood pressure in doses from 0 1 mg/kg up to totally 7 mg/kg (fig 13 table 4) After the doses of 8 and 16 mg/kg (total doses 15 and 31 mg kg) cardiac output and arterial blood pressure were markedly reduced, while left atrial blood pressure increased One dog died after the dose of 8 mg kg and the remaining four during the infusion of 16 mg/kg

Propranolol, in a dose of 0 1 mg/kg, significantly reduced basal aortic flow and heart rate (fig 13, table 4) The additional doses of 1 0, 2 0 and 4 0

c) Effects on cardiac responses to calcium chloride, noradrenaline and peripheral vagal or sympathetic nerve stimulation

The influence of H 56/28 or propranolol (4 mg/kg i v) on cardiovascular responses to intravenously injected calcium chloride and noradrenaline and to electrical stimulation of the peripheral parts of the vagal and sympathetic nerves were studied in barbiturized cats

Bilateral vagotomy was performed in the neck The preganglionic fibres to the right stellate ganglion were cut Heart rate, cardiac contractile force and mean arterial blood pressure were recorded

At 15 minute intervals control responses to intravenously injected calcium chloride (10 mg/kg) and noradrenaline (0 5 µg/kg) and to stimulation of the peripheral part of the right vagal nerve (stimulated for 15 seconds with 2–5 impulses/sec , 2 msec impulse duration and supramaximal voltage) and the right stellate ganglion (stimulated for 15 seconds with 10 impulses/sec , 2 msec impulse duration and supramaximal voltage) were obtained

After recording the control responses H 56/28 or propranolol was injected intravenously over two minutes in a dose of 4 mg/kg Ten minutes later calcium chloride was given followed by noradrenaline stimulation of vagal nerve and stellate ganglion at 15 minute intervals as carried out previously during the determination of the control responses

Four experiments were performed with each antagonist Two control experiments were carried out where physiological saline was given instead of an inhibitor In these control studies no significant changes occurred in the responses to the tested stimuli

The results are shown in fig 12 The positive chronotropic and inotropic effects of sympathetic nerve stimulation and of injected noradrenaline were almost completely blocked by both antagonists Neither H 56/28 nor propranolol significantly altered the positive inotropic effect of calcium chloride the negative chronotropic effect produced by vagal stimulation or the pressor effect of noradrenaline

4 *Effects of H 56/28 and propranolol on aortic blood flow and arterial and left atrial blood pressure in the anaesthetized dog*

The effects of H 56/28 and propranolol on basal general hemodynamics and on the cardiovascular responses to intravenously infused isoprenaline *were studied in anaesthetized dogs*

H 56/28 or propranolol was given intravenously in increasing doses of 0 1 1 0 2 0 4 0 8 0 and 16 0 mg/kg All doses were given by constant infusion over ten minutes with an *interval of 30 minutes (between the doses of 0 1* and 1 0 mg kg and between 1 0 and 2 0 mg/kg) or ten minutes (between the higher doses)

The cardiovascular effects of isoprenaline, infused intravenoulsy for two minutes in a dose of 0 3 µg/kg and minute were studied three times before administration of the antagonist minutes after the first *(0 1 mg/kg) and the second* (1 0 mg kg) dose of the antagonist had been given

Five experiments were performed with each antagonist Three control experiments were performed where physiological saline was given instead of an antagonist

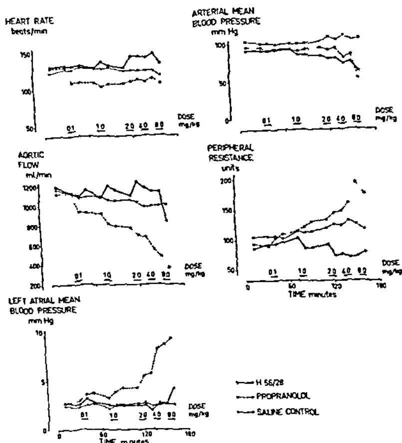


Fig 13 Dogs anaesthetized with morphine, diallymal and urethane Heart rate, aortic flow left atrial and arterial mean blood pressure and peripheral vascular resistance recorded. Effects of H 56/28 or propranolol, administered i v in increasing doses as indicated Mean results of five experiments with each antagonist and three control experiments

mg/kg gave some further decrease of aortic flow, mainly due to reduced stroke volume These effects were accompanied by increases of left atrial blood pressure and peripheral vascular resistance, while the arterial mean blood pressure was only slightly reduced

In doses of 8 and 16 mg/kg (total doses 15 and 31 mg/kg) propranolol markedly reduced cardiac output and arterial blood pressure, while left atrial blood pressure and peripheral vascular resistance increased Two dogs died after the administration of the dose of 8 mg/kg and the remaining three died during the infusion of the dose of 16 mg/kg

The ECG recordings showed that all dogs had sinus rhythm which persisted until the agonal phase After 0 1 mg/kg propranolol the PR interval was

Table 3 Cardiovascular effects of isoprenaline in anaesthetized dogs before and after administration of H 56/28 and propranolol The values represent the effects recorded at the end of the isoprenaline infusion Mean values and s e m from five experiments with H 56/28 and propranolol and three control experiments

Parameter	Substance	Effect of isoprenaline			Blockade of isoprenaline response	
		before drug	after drug 0 1 mg/kg	after drug 0 1+1 0 mg/kg	after drug 0 1 mg/kg	after drug 0 1+1 0 mg/kg
Heart rate beats/min	H 56/28	+ 78± 6 2	+ 25±7 5	+ 4±0 7	69±8 2%	95±1 3%
	Propranolol	+ 62±11 7	+ 22±4 4	+ 4±1 2	65±3 0%	94±1 4%
	Saline control	+ 78± 2 4	+ 78±8 1	+ 86±5 8		
Aortic flow ml/min	H 56/28	+937±238	+371±101	+ 80±34	64±7 5%	91±2 5%
	Propranolol	+624± 71	+281± 47	+ 59±14	67±7 5%	91±1 9%
	Saline control	+967±304	+866±360	+974±351		
Arterial mean blood pressure mm Hg	H 56/28	— 12±4 9	— 5±2 3	— 1±1 2		
	Propranolol	— 22±4 4	— 4±1 4	— 1±1 6		
	Saline control	— 21±2 9	— 14±0	— 6±2.2		
Peripheral resistance units	H 56/28	— 41±5 4	— 22±6 4	— 6±3 1	49±8 1%	86±5 9%
	Propranolol	— 50±12	— 29±3 7	— 13±4 7	36±9 3%	77±7 9%
	Saline control	— 61±24	— 52±18	— 58±26		
Left atrial mean blood pressure mm Hg	H 56/28	—1 1±0 06	—0 9±0 28	± 0±0 14		
	Propranolol	—1 5±0 35	—1 3±0 39	—0 2±0 18		
	Saline control	—0 9±0 26	—1 5±0 49	—1 5±0 41		

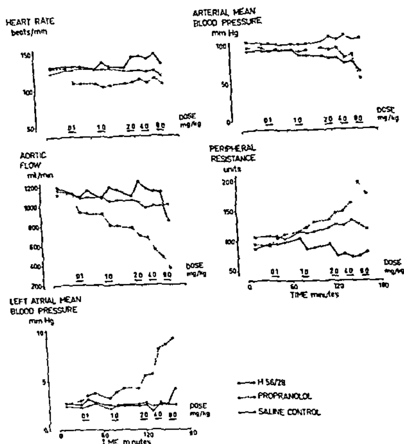


Fig. 13 Dogs anaesthetized with morphine, diallymal and urethane Heart rate, aortic flow, left atrial and arterial mean blood pressure and peripheral vascular resistance recorded Effects of H 56/28 or propranolol, administered i.v in increasing doses as indicated Mean results of five experiments with each antagonist and three control experiments

mg/kg gave some further decrease of aortic flow, mainly due to reduced stroke volume These effects were accompanied by increases of left atrial blood pressure and peripheral vascular resistance, while the arterial mean blood pressure was only slightly reduced.

In doses of 8 and 16 mg/kg (total doses 15 and 31 mg/kg) propranolol markedly reduced cardiac output and arterial blood pressure, while left atrial blood pressure and peripheral vascular resistance increased Two dogs died after the administration of the dose of 8 mg/kg and the remaining three died during the infusion of the dose of 16 mg/kg

The ECG recordings showed that all dogs had sinus rhythm which persisted until the agonal phase After 0 1 mg/kg propranolol the PR interval was

Table 4. Cardiovascular effects of H 56/28 and propranolol in anaesthetized dogs. Initial levels and changes recorded ten minutes after i.v. infusions of the drugs in doses of 0.1 and 1.0 mg/kg. Mean values and s.e.m. from five experiments with H 56/28 and propranolol and three control experiments

Parameter	Substance	Initial value	Change after drug 0.1 mg/kg	Change after drug 0.1+1.0 mg/kg
Heart rate beats/min	H 56/28	131±16	+ 0±8.4	− 1±11
	Propranolol	128±11	−21±4.9	−23±8.8
	Control	126±18	+ 2±7.1	+ 1±3.6
Aortic flow ml/min	H 56/28	1118±211	+40±79	+40±94
	Propranolol	1122±180	−202±37	−344±55
	Control	1122±266	−37±71	−78±21
Arterial mean blood pressure mm Hg	H 56/28	91±9.2	± 0±3.2	− 8±4.4
	Propranolol	94±7.2	− 2±3.4	− 3±3.4
	Control	100±4.4	− 2±0.2	− 1±1.0
Peripheral resistance units	H 56/28	92±18	+ 3±5.7	− 5±8.4
	Propranolol	90±12	+20±7.6	+38±13
	Control	106±34	− 4±5.8	+ 8±5.9
Left atrial mean blood pressure mm Hg	H 56/28	2.5±0.42	+0.2±0.17	−0.2±0.32
	Propranolol	3.0±0.47	+0.7±0.24	+1.3±0.42
	Control	2.2±0.24	+0.3±0.24	+0.3±0.20

increased by about 10% simultaneously with the reduction of the heart rate. Otherwise no changes in the ECG pattern were noted until after the doses of 4 and 8 mg/kg. Then a prolonged PR interval and QRS time, an elevated ST segment and an increased T positivity were seen both after H 56/28 and propranolol. These changes became more marked when 16 mg/kg were given of the two compounds. None of these ECG changes were seen in the control experiments.

Discussion

The effects of H 56/28 observed in the present experiments can be explained on the basis of *three* fundamental pharmacodynamic actions of the drug.

The *first* and predominant action of H 56/28 is *blockade of β-adrenergic receptors*, as defined by AHLQUIST (1948). H 56/28 inhibited positive cardiac inotropic and chronotropic responses to isoprenaline, noradrenaline and to sympathetic nerve stimulation. Further H 56/28 antagonized isoprenaline-induced relaxation of vascular and tracheal smooth muscle.

The studies of contractile force in isolated papillary muscle showed that

treatment with H 56/28 gave a parallel shift to the right of the cumulative isoprenaline dose response curve indicating a blockade of the competitive type In the same preparation the positive inotropic effects of ouabain calcium chloride and theophyllamine considered to be due to mechanisms other than β-receptor activation (cf MORAN & PERKINS 1958 RALL & WEST 1963 LEVY & RICHARDS 1965*a*) were not inhibited by H 56/28 even in concentrations 100 times higher than those giving pronounced blockade of the isoprenaline response This finding indicates that H 56/28 is a highly selective β receptor antagonist The same conclusion can be drawn from the in vivo studies In cats doses of H 56/28 giving pronounced β-receptor blockade did not significantly affect the positive inotropic effect of calcium chloride on heart the negative chronotropic effect of vagal nerve stimulation or the pressor response to noradrenaline In studies on dogs where blood flow in a hind leg was recorded H 56/28 (1 0 mg/kg i v) was found to inhibit the vasodilator effect of isoprenaline injected into the femoral artery whereas the vasodilator responses to intra arterially injected histamine and acetyl choline and noradrenaline induced vasoconstriction were unchanged (unpublished observations)

The present results with propranolol are in agreement with previously reported data (BLACK *et al* 1964 1965) indicating that this compound is a highly selective β-receptor antagonist H 56/28 and propranolol were found to be approximately equipotent antagonists in all studies performed When administered intravenously to cats the two agents had similar time-effect curves characterized by rapid onset maximum 5–10 minutes after administrat on and duration of more than two hours after a dose of 0 1 mg/kg

The laevo isomer of H 56/28 was a more potent antagonist than the racemate while the dextro isomer had very little activity as regards the inhibition of the positive chronotropic effect of isoprenaline the approximate ratio between equipotent doses of the three forms of H 56/28 was found to be 1 2 100 respectively The β-receptor blocking activities of the two optical isomers and the racemate of propranolol are characterized by a similar relationship (HOWE & SHANKS 1966)

Another action of H 56/28 was found in the experiments on reserpinized cats where the drug produced positive card ac chronotro[illegible] effects [illegible] inhibite[illegible] the sec[illegible] direct β receptor stimulation Propranolol has previously been shown to be devoid of such an action (BLACK *et al* 1965)

The cardiac β receptor stimulating and blocking actions of H 56/28 were characterized by the same time curve in cats The dose-response curves for the two actions differed however The heart stimulation reached its maximum

after 0.05–0.15 mg/kg H 56/28 i.v.; higher doses gave no further cardiac stimulation but an increasing dose-dependent β-blockade.

The studies on the optical isomers of H 56/28 indicate that the β receptor stimulating *activity of the racemate, like its β-receptor blocking action,* is mainly exerted by the laevo isomer. The heart stimulating action of H 56/28 is not limited to reserpinized cats but can also be demonstrated in cats where the endogenous sympathetic tone is abolished by hexamethonium treatment (JOHNSSON *et al.* 1966), and in isolated rabbit hearts, especially those from reserpinized animals (unpublished observations).

H 56/28 has also been found to have a weak stimulating effect on vascular β-receptors (EK & JOHNSSON, unpublished observations): in reserpinized dogs 0.02 mg H 56/28 given via a femoral artery produced a small decrease of the peripheral vascular resistance in the hind leg. This effect developed gradually over about 5 minutes and could be inhibited by propranolol in doses (0.2 *mg intraarterially or 0.1 mg/kg i.v.) which per se did not influence basal blood* flow in the region.

All these findings justify the conclusion that H 56/28 is a β-adrenergic receptor antagonist possessing a weak β-adrenergic "intrinsic activity" (cf. ARIËNS *et al.* 1964). From the viewpoint of receptor theory H 56/28 should accordingly be classified as a "partial agonist" (STEPHENSON 1956) or a "competitive dualist" (ARIËNS *et al.* 1964).

Higher doses of H 56/28 (from 0.5–1.0 mg/kg) reduced cardiac rate and contractility in reserpinized cats. This finding demonstrates a *third* fundamental action of the drug: *a direct depression of cardiac function.* This action in several respects was found to differ from the β-receptor blocking and stimulating actions of the drug: 1) It was of significant importance only in doses considerably above those producing marked β-receptor blockade, 2) it was characterized by a much shorter duration than the β-receptor blockade, 3) *the laevo and dextro isomers of H 56/28 were equally active in producing* direct cardiac depression while their β-blocking activities were widely different.

Propranolol was found to elicit a direct cardiac depressant effect of the same type as H 56/28 and it was equipotent to H 56/28 in this respect. Several authors have shown that high doses of propranolol elicit a cardiac depressant action which is a direct effect and not due to β-receptor blockade (LEVY & RICHARDS 1965*b*, MCINERNY *et al.* 1965, MORALES-AGUILERA & VAUGHAN WILLIAMS 1965).

There is evidence that higher doses of propranolol and H 56/28 may elicit a direct depressant action not only in cardiac muscle but also in smooth muscle. JOHNSSON (1967) found a small transient vasodilatation in the human forearm during intra-arterial infusion of propranolol or the racemic or dextro forms of H 56/28 in a dose of 0.5 mg, while propranolol and racemic H 56/28

in a dose of 0.05 mg produced a marked and long lasting vascular β-receptor blockade. Similar observations were made when these agents were given intra arterially in the hind leg of reserpinized dogs (EK & JOHNSSON unpublished observations). Intra arterially injected propranolol has previously been reported to elicit a transient vasodilator action in man (SCHOOP & SCHMIDTKE 1965) and dog (NAKANO & KUSAKARI 1965, 1966, SHANKS 1967). —In the present study on tracheal smooth muscle a reduction of smooth muscle tension was elicited by propranolol and H 56/28 in concentrations about 50 times higher than those producing 80% blockade of isoprenaline-induced relaxation, it was found that the laevo and dextro isomers of H 56/28 were equally active in producing this depression (unpublished observations).

The mechanism for the direct cardiac and smooth muscle depressant action of H 56/28 and propranolol is not known. The cardiodepressant effect of the two agents was not influenced by atropine pretreatment in the present experiments and NAKANO & KUSAKARI (1965) likewise reported that atropinization did not affect the vasodilator response to propranolol. Thus these actions were probably not due to activation of cholinergic receptors. Propranolol has been found to exert a quinidinelike depression of cardiac membrane depolarization (MORALES AGUILERA & VAUGHAN WILLIAMS 1965) and it has been suggested that this action may also involve a depression of contractility through interference with the excitation contraction coupling mechanism (LEVY & RICHARDS 1965*b*). The direct cardiac and smooth muscle depressant action of these agents may further be related to their local anaesthetic action. The laevo and dextro isomers of H 56/28 have been reported to be approximately equally active local anaesthetic agents, the activity of the two isomers and of propranolol was further found to be in the same range as that of lidocaine (ÅKERMAN personal communication 1967). It is well known that local anaesthetic agents can depress cardiac contractility and smooth muscle tone (TRUANT & TAKMAN 1965).

Some observations do, however, not reconcile with the hypothesis that the direct cardiodepressive and local anaesthetic actions of β-receptor antagonists are interrelated. The β-receptor blocking agents MJ 1999 (LISH *et al* 1965) and INPEA (SOMANI & LUM 1965) are reportedly devoid of local anaesthetic properties. In studies on isolated mammalian heart muscle preparations MCINERNY *et al* (1965) found however, that the depressant activity of MJ 1999 did not differ from that of propranolol. We found that both MJ 1999 and INPEA produced a cardiodepressive action in reserpinized, vagotomized and adrenalectomized cats, the effect beginning to appear at an i.v. dose of 1–2 mg/kg (unpublished observations).

Since the direct cardiac and smooth muscle depressant actions of H 56/28 and propranolol were found to be of significant importance only after intravenous doses above 1 mg/kg, the integrated cardiovascular response to lower

after 0 05–0 15 mg/kg H 56/28 i v, higher doses gave no further cardiac stimulation but an increasing dose-dependent β-blockade

The studies on the optical isomers of H 56/28 indicate that the β receptor stimulating activity of the racemate, like its β-receptor blocking action is mainly exerted by the laevo isomer The heart stimulating action of H 56/28 is not limited to reserpinized cats but can also be demonstrated in cats where the endogenous sympathetic tone is abolished by hexamethonium treatment (JOHNSSON *et al* 1966), and in isolated rabbit hearts, especially those from reserpinized animals (unpublished observations)

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All these findings justify the conclusion that H 56/28 is a β adrenergic receptor antagonist possessing a weak β-adrenergic 'intrinsic activity' (cf ARIËNS *et al* 1964) From the viewpoint of receptor theory H 56/28 should accordingly be classified as a "partial agonist" (STEPHENSON 1956) or a "competitive dualist" (ARIENS *et al* 1964)

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In the studies on cats and dogs the arterial blood pressure was not significantly influenced by H 56/28 or propranolol in i v doses below 1 mg/kg High doses of both drugs produced hypotension, probably mainly due to the direct cardiodepressant action In the dog study propranolol produced an increase of the peripheral vascular resistance already in low doses This effect could be due partly to a reflex increase of vasoconstrictor nerve activity secondary to the reduction of cardiac output, partly to a blockade of endogenous tone on vascular β-receptors

In the dog study a marked decrease of cardiac output with severe ECG disturbances, was observed after H 56/28 or propranolol in a total dose of 7–15 mg/kg These effects were probably due to the direct cardiodepressant action of the drugs On the other hand the two agents were found to produce marked β-receptor blockade already in a dose of 0 1 mg/kg These results support the above made conclusion that the direct cardiodepressant action of the antagonists is of hemodynamic importance only after doses much higher than those producing marked β receptor blockade

The acute and chronic toxicity of H 56/28 has been investigated in several species (HANSSON & MAGNUSSON personal communication 1967) The acute LD 50 was about 100 times higher than the dose giving marked β-receptor blockade Lethal doses produced convulsions and cyanosis before death The acute LD 50 of the laevo and dextro isomers of H 56/28 were found to be similar This indicates that death could be ascribed to the local anaesthetic and direct cardiac depressive actions rather than to β receptor blockade

All studies performed with H 56/28 so far indicate that there is a wide margin between the dose that gives β receptor blockade and the dose that gives overt toxic symptoms In unanaesthetized dogs an oral dose of 1 mg/kg produced marked β-receptor blockade whereas the behaviour of the dogs was influenced only by oral doses above 60 mg/kg

The chronic toxicity of H 56/28 was evaluated in dogs and rats which received high doses orally for six months In this investigation, which included detailed studies as regards hematology, blood chemistry, urine analysis and pathological anatomy no unexpected toxic effects were observed No carcinogenic effects were observed in mice treated with H 56/28 for more than one year

Human pharmacological studies of H 56/28 (JOHNSSON *et al* 1966 1967, FORSBERG & JOHNSSON 1967, ÅBLAD *et al* 1967) have yielded results that are in good agreement with the animal data FORSBERG and JOHNSSON (1967) compared the hemodynamic effects of H 56/28 (10 mg i v) and propranolol (10 mg i v) in healthy subjects under resting conditions The two agents were found to produce equal blockade of the cardiovascular response to intravenously infused isoprenaline Resting cardiac output was consistently reduced after propranolol by 22% on the average After H 56/28, however,

doses of these drugs should only be due to their β receptor blocking properties The response to H 56/28 should be modified, however, by its weak β-receptor stimulating action.

H 56/28 and propranolol were found to antagonize the cardiac effects of *sympathetic nerve stimulation in cats but an i v dose of 0 4 mg/kg* was needed of produce 70–80% blockade In these experiments the stellate ganglion was stimulated with a frequency of 10 impulses/second which corresponds to the highest physiologically occurring discharge rate in the cardiac sympathetic nerves (UVNÄS 1960) The comparatively low basal heart rates observed in the nonreserpinized cats indicate that the endogenous discharge frequency in their sympathetic nerves was much lower than 10 impulses/second *Such a low cardiac sympathetic tone could be expected to be almost* eliminated by an i v dose of 0 05 mg/kg of either propranolol or H 56/28 due to their β-receptor blocking action

These considerations are supported by the present experiments on nonreserpinized cats A significant reduction of heart rate and cardiac contractile force was seen after an i v dose of 0 05 mg/kg of propranolol and higher doses, up to 2 mg/kg, did usually not produce much further change

H 56/28 elicited a cardiac β-receptor stimulating effect in an i v dose of 0 05 mg/kg with little further change after higher doses This action should consequently counteract the negative cardiac chronotropic and inotropic effects due to the inhibitory action of the drug on endogenous sympathetic tone In most experiments on non-reserpinized cats, basal cardiac rate and contractile force were practically unchanged after *H 56/28* in i v doses from 0 05 to 2 mg/kg, indicating that the prevailing endogenous cardiac sympathetic tone corresponded quantitatively to the cardiac stimulating effect of the drug

In situations with a high cardiac sympathetic tone, on the other hand H 56/28 could be expected to reduce cardiac rate and contractility since its β receptor blocking action should then be of dominating importance This is probably examplified by the negative chronotropic and inotropic effects seen *after 0 05 and 0 3 mg/kg H 56/28 in cats with a high basal heart rate* However, even in these animals the cardiac β-receptors should remain moderately activated after H 56/28 treatment because of the sympathomimetic action of the drug

In the study on anaesthetized dogs propranolol in i v doses of 0 1 and 1 0 mg/kg, reduced cardiac output and *heart rate and increased mean left* atrial pressure These effects were probably due to inhibition of endogenous cardiac sympathetic tone through β receptor blockade Such hemodynamic changes were not seen after the same doses of H 56/28 This agent probably blocked endogenous cardiac sympathetic tone to the same degree as pro pranolol, but this inhibitory effect of H 56/28 was *counterbalanced by its* β receptor stimulating effect on the heart

to 2 mg/kg In the same preparations propranolol consistently elicited negative cardiac chronotropic and inotropic effects already in an i v dose of 0 05–0 1 mg/kg presumably because of unopposed elimination of endogenous sympathetic tone on heart

6 H 56/28 and propranolol were further found to possess a direct cardiodepressant action This action was of importance for the integrated response to the drugs only at a dose level considerably above that giving marked β-receptor blockade

7 The β-receptor blocking and stimulating actions of H 56/28 were predominantly exerted by its laevo isomer The approximate ratio between equipotent β-receptor blocking doses of racemic, laevo and dextro H 56/28 was 2 1 100 respectively The laevo and dextro isomers of H 56/28 were equally active in producing direct cardiac depression

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The authors wish to thank Miss Ann Christin Andersson, Mrs Gunilla Friberg Mrs Gull-Britt Henning Miss Eva Hultberg, Miss Annelise Meul, Miss May Svenningsson and Mrs Lisbeth Zackrisson for excellent technical assistance, Mr Elof Gustafsson for the skilful construction of the strain gauge arches and other experimental equipment, Mr Roy Hartmark for his supply of experimental animals in good condition and Miss Marianne Bergbrant for efficient secretarial aid

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cardiac output was not significantly changed These results indicate that also in man, the inhibitory effect of H 56/28 on resting cardiac sympathetic drive is counterbalanced by its slight sympathomimetic action on heart

Hitherto reported clinical studies with H 56/28 indicate that the drug is an active and well tolerated β-receptor antagonist with therapeutic value in cardiac arrhythmias (LINKO *et al* 1967) and angina pectoris (BJÖRNTORP 1967)

The experience from the use of propranolol in patients with cardiac disease indicates that the most important undesirable effect of this drug is cardiac depression due to elimination of cardiac sympathetic drive (STEPHEN 1966) Such a complication should be expected to occur less frequently in the clinical use of H 56/28 because its β-receptor blocking effect is combined with a weak heart stimulating action This question is now being investigated

Summary

1 The activity of the compound H 56/28 (1-(o-allyl-phenoxy)-3-isopropyl-amino-2-propranol hydrochloride) was studied in various animal preparations *in vitro* and *in vivo* The main object of the study was to analyze the mechanisms of the cardiovascular actions of the drug The effects of H 56/28 were compared to those of propranolol

2 The predominant action of H 56/28 was β-adrenergic receptor blockade In isolated papillary muscle H 56/28 produced competitive inhibition of the positive inotropic response to isoprenaline

In anaesthetized cats or dogs H 56/28 inhibited the positive cardiac chronotropic and inotropic responses to isoprenaline or to electrical stimulation of the cardiac sympathetic nerves, and the drug also blocked the hypotensive and vasodilator effect of isoprenaline

Doses of H 56/28 giving marked β-receptor blockade, did not significantly influence the inotropic effect of ouabain, theophyllamine or calcium chloride on heart the negative chronotropic effect of peripheral vagal nerve stimulation or the vasoconstrictor response to noradrenaline

3 The β-receptor blocking activity of H 56/28 was equal to that of propranolol When given intravenously to cats the two compounds were found to have similar time effect curves

4 The β-receptor blocking action of H 56/28 was combined with a weak direct β-receptor stimulation In reserpinized cats H 56/28 produced moderate stimulation of cardiac rate and contractile force This effect could be inhibited by propranolol, the latter agent being devoid of cardiac stimulating properties

5 The weak β-receptor stimulating action of H 56/28 modified its integrated cardiovascular response in anaesthetized cats and dogs with endogenous cardiac sympathetic tone In such preparations H 56/28 did not as a rule influence basal cardiac rate contractile force or output in i v doses from 0 05

to 2 mg/kg In the same preparations propranolol consistently elicited negative cardiac chronotropic and inotropic effects already in an i v dose of 0 05–0 1 mg/kg, presumably because of unopposed elimination of endogenous sympathetic tone on heart

6 H 56/28 and propranolol were further found to possess a direct cardiodepressant action This action was of importance for the integrated response to the drugs only at a dose level considerably above that giving marked β-receptor blockade

7 The β-receptor blocking and stimulating actions of H 56/28 were predominantly exerted by its laevo isomer The approximate ratio between equipotent β-receptor blocking doses of racemic, laevo and dextro H 56/28 was 2 1 100 respectively The laevo and dextro isomers of H 56/28 were equally active in producing direct cardiac depression

Acknowledgements

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From AB Hässle, Department of Pharmacology Goteborg Sweden

The Effect of Propranolol and the dextro and laevo Isomers of H 56/28 upon Ouabain-Induced Ventricular Tachycardia in Unanaesthetized Dogs

By

B R DUCE[1], L GARBERG and B JOHANSSON[2]

The occurrence of cardiac arrhythmias due to digitalis intoxication is a major clinical problem Experience has shown that beta adrenergic blocking agents suppress certain types of arrhythmias including those resulting from overdigitalization (STOCK & DALE 1963, TAYLOR *et al* 1964, GINN *et al* 1965 LINKO *et al* 1966, BOIS *et al* 1966) Animal experiments have indicated *that blockade* of the beta receptors does not entirely explain abolishment of this type of arrhythmia (LUCCHESI & HARDMAN 1961, SOMANI & LUM 1965, LUCCHESI 1965) This contention is supported by *recent* evidence that the weak beta blocking dextro isomers of pronethalol (HOWE 1965) and *propranolol* (HOWE & SHANKS 1966) can *revert* ouabain induced arrhythmia LUCCHESI (1965) has shown this effect in dogs with dextro pronethalol and HOWE & SHANKS (1966) have abolished ouabain induced arrhythmia in cats *using* dextro propranolol

ÅBLAD *et al* (1967) have concurrently *reported* the pharmacology of a new beta adrenergic antagonist H 56/28 They also investigated *the optical* isomers of this compound *The laevo* isomer of H 56/28 was found to be twice as active as the racemic form of propranolol in blocking beta ...

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with ... more potent. H 56/28, its isomers and propranolol are about equally cardiodepressive in doses which greatly exceed their beta adrenergic receptor blocking requirements

The purpose of the present investigation was to study *the possible* anti-

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Table 1

Treatment	Exp nr	Dog body weight (kg)	Cumul ouabain dose µg/kg	Heart frequency beats/min: Mean initial	Tachy-cardia before treatment	Lowest average after clearing	Effect of treatment on ventricular arrhythmia: Onset time of complete clearing (min)	Duration of complete clearing (min)
Saline	81	10 8	70	104	247		no effect	
	82	12 1	60	108	258		no effect	
	83	11 5	60	153	189		no effect	
	89	15 6	60	114	204		no effect	
	90	10 5	70	107	231		no effect	
	91	11 6	60	148	216		no effect	
	92	11 4	60	118	249		no effect	
	94	14 9	60	166	220		no effect	
Mean ± s e m		12 3±0 7	62 5±1 6	127±9	227±8			
Propranolol	130	14 3	60	114	222		no effect	
	161	7 5	70	156	217		no effect	
	114	12 6	60	90	240	22	12	>78
	124	12 5	60	105	219	32	immediate	>90
	146	9 8	60	99	237	26	24	32
	153	10 7	70	146	183	65	4	24
Mean ± s e m		11 2±1 1	63 3±2.3	118±11	220±8			
Mean ± s e m Effective exps				110±12	220±14	36±10		
H 56/28 laevo	119	9 4	60	91	226		no effect	
	125	11 6	90	130	221		partial clearing	
	147	8 6	70	109	230		partial clearing	
	175	17 0	60	99	218	38	12	74
	128	11 5	70	170	224	115	12	>48
	159	11 6	70	106	231	94	immediate	10
Mean ± s e m		11 6±1 2	70 0±4 5	118±12	225±2			
Mean ± s e m Effective exps				125±23	224±4	82±23		
H 56 28 dextro	127	9 4	70	126	235		no effect	
	116	9 8	60	132	194	71	immediate	>90
	121	8 7	70	80	189	50	immediate	>90
	148	8 6	60	139	188	60	immediate	>90
	160	10 6	70	75	199	45	immediate	>90
	165	8 5	70	131	204	40	immediate	>90
Mean ± s e m		9 3±0 3	66 7±1 9	114±12	202±7			
Mean ± s e m Effective exps				111±14	195±3	53±6		

The effect of

arrhythmic activity of the isomers of H 56/28 in comparison with propranolol on ouabain-induced ventricular tachycardia in the unanaesthetized dog

Material and Methods

Thirty five unanaesthetized untrained adult mongrel dogs of either sex weighing between 7 5 and 17 kg were used The dogs were slightly restrained in the left lateral recumbent position during the experiments The right saphenous vein was cannulated for injection purposes

Electrocardiograms were recorded with an Offner type R oscillograph using a CF lead with a surface electrode on the right thorax over the fourth intercostal space about fifteen centimeters from the spine The clear P waves in this lead facilitated the analysis of the cardiac rhythm The electrocardiogram was also monitored on a cathode ray oscilloscope

After a ten minute accommodation period ouabain was administered in divided doses until persistent ventricular tachycardia was induced The initial dose was 0 04 mg/kg followed by 0 02 mg/kg thirty minutes later Additional 0 01 mg/kg doses were given at successive thirty minute intervals if required The test substances or physiological saline were injected over two minutes when the tachycardia had been established for five minutes

Electrocardiograms were taken every five or ten minutes during the accommodation period and after ouabain until ventricular ectopics appeared and then every fourth minute following the injection of the test substance or saline The first twenty seconds of each record were used for measuring the heart rate and analysing the ventricular beats which were classified as either atrial or idioventricular in origin

The mean initial heart rates given in the results represent an average of the three consecutive values obtained prior to ouabain administration

The effect of the test substances or saline on the digitalis induced ventricular tachycardia was classified as 1 *no effect* ventricular tachycardia persisting for 30 minutes or more following injection 2 *partial clearing* reduction of the heart rate to below the mean initial value but with residual ventricular ectopics exceeding five per cent of the total beats 3 *complete clearing* abolishment of ventricular ectopic frequency to less than five per cent of the total beats The duration of antiarrhythmic activity was estimated in cases of complete clearing by continuing the electrocardiographic observations for ninety minutes after treatment (sixty minutes in one dog)

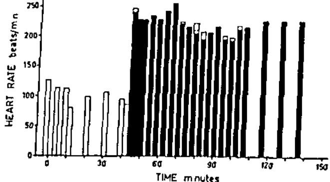
In order to assess the bradycardia which often followed conversion from ventricular tachycardia the lowest average heart rate of any three consecutive samples taken within one hour of treatment was recorded

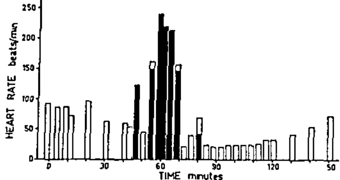
The results were analyzed for statistical significance using Student s *t* test and Fischer s exact probability test Variability was expressed as the standard error of the mean

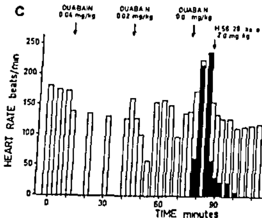
The hydrochloride salt of propranolol and H 56/28 dextro was used whereas H 56 28 laevo was in the tartrate form The H 56/28 laevo preparation contained less than 0 2% of the dextro isomer and H 56/28 dextro contained less than 1 0% of the laevo form (BRANDSTROM personal communication 1966) Aqueous solutions were prepared on the day of the experiment and all doses were calculated on the basis of the hydrochloride

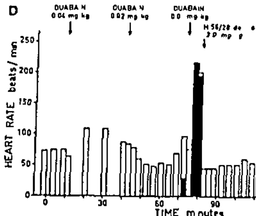
Results

The initial heart rates were relatively high in all of the experiments due to the fact that untrained animals were used There was no significant difference in these values between the groups studied (Table 1)









☐ Normal beats ■ Ventricular ectopic beats ☐ Questionable beats

Fig. 1. The effect of (A) physiological saline and 2.0 mg/kg of (B) propranolol, (C) H 56/28 laevo or (D) H 56/28 dextro upon ouabain induced ventricular tachycardia in the unanesthetized dog. All substances injected intravenously over two minutes.

The cumulative dose of ouabain required to induce persistent ventricular tachycardia was obtained from the thirty-five animals used in this study Eighteen dogs required 0 06 mg/kg, sixteen required 0 07 mg/kg and one required 0 09 mg/kg, resulting in a mean value of 0 065 mg/kg The amount of ouabain required did not differ significantly between the groups receiving the various substances

Recurrent vomiting invariably occurred following administration of the glycoside Persistent ventricular tachycardia usually occurred abruptly after the required accumulative dose of ouabain had been injected Sometimes, however, the tachycardia was preceded by occasional or short trains of ventricular ectopics

Eight dogs in the study served as a control group and received only physiological saline following the occurrence of ventricular tachycardia The results demonstrated that once the arrhythmia had been established for five minutes it would persist uninterrupted In seven out of the eight experiments the duration of the tachycardia was over ninety minutes The other animal reverted to sinus rhythm after forty minutes. The results obtained in the control group are summarized in the first section of Table 1 and one representative experiment is shown in detail in Fig 1A

The remaining twenty-seven dogs were treated with propranolol, H 56/28 dextro, or H 56/28 laevo after development of ventricular tachycardia Doses of 1 mg/kg or 2 mg/kg were used

Three experiments were carried out with each of the test substances at a dose of 1 mg/kg Of the three dogs receiving 1 mg/kg propranolol one showed no effect, one showed partial clearing and the third one showed a twenty minute period of complete clearing beginning twenty-four minutes after the drug injection In the group with 1 mg/kg H 56/28 laevo one showed no antiarrhythmic effect, one showed partial clearing and one showed complete clearing for more than ninety minutes The same dose of H 56/28 dextro was ineffective in two dogs and resulted in partial clearing in a third

Since all three test substances showed an inconsistent effect on the ventricular arrhythmia after 1 mg/kg, the study was expanded with additional experiments using the 2 mg/kg dose level Six dogs were used with each of the drugs

The results presented in Table 1 show that of the dogs treated with propranolol two out of six showed no effect on the ventricular arrhythmia The other four had periods of complete clearing with the onset of action varying from immediate to twenty-four minutes after injection Sinus rhythm persisted for the duration of the experiment in two of these animals In the other two dogs ventricular tachycardia returned twenty-four and thirty-two minutes after clearing The occurrence of clearing in four out of six animals in this group indicates a statistically significant difference from the eight saline controls ($p=0.025$) A pronounced bradycardia characterized the clearing

effect is approximately one hundred times weaker (ÅBLAD *et al* 1967) This lack of correlation between antiarrhythmic and beta adrenergic blocking activity was demonstrated by LUCCHESI (1965) with pronethalol and its dextro isomer, and with propranolol and its isomers (HOWE & SHANKS, 1966) SOMANI and LUM (1965) have suggested that the antiarrhythmic effect of pronethalol and related agents is associated with local anaesthetic action This explanation could be relevant in the present study since both isomers of H 56/28 have this property (ÅKERMAN personal communication 1966)

Following reversion of ouabain induced ventricular tachycardia by H 56/28 dextro or propranolol there was a consistent slowing of the heart rate below the initial values This may be ascribed to the unmasking of digitalis induced depression of the sinus node and, in the case of propranolol, to sympathetic blockade as well The fact that pronounced bradycardia did not occur in those animals which cleared after H 56/28 laevo is possibly explained by the slight sympathomimetic effect of this drug In three out of the four successful experiments with 2 0 mg/kg of propranolol, the heart rate fell below forty beats per minute after conversion to sinus rhythm This value is the lowest basal rate that we have observed in trained untreated dogs The heart frequency did not fall below this level in any of the dogs given 2 0 mg/kg of H 56/28 dextro

In treating digitalis-induced ventricular tachycardia there is *some risk of* ventricular asystole since the abolition of the idioventricular rhythm may unmask other aspects of digitalis toxicity namely sinus depression and A–V blockade (GOLDBERG & COTTEN 1951) Antiarrhythmic drugs which exert a negative chronotropic effect, for instance, by blocking the sympathetic control of the heart may be especially hazardous in this respect The dextro isomer of H 56/28 which is essentially devoid of beta adrenergic blocking action has shown to effectively suppress ventricular arrhythmias induced by digitalis *in this study*

The present results allow no generalizations about the possible effectiveness of the dextro isomer of H 56/28 in cases of clinical digitalis *intoxication* or *in* experimental and clinical arrhythmias of other kinds It is not known whether the positive results that have been reported with beta adrenergic blocking agents on rhythmic heart disturbances in clinical practice should be ascribed to the adrenergic blockade or to other effects that "active" and "inactive" isomers may have in common The recent results by LINKO *et al* (1967 and personal comm [illegible]
however They ga [illegible]
in doses of 4 to [illegible] patients with different, mainly acute arrhythmias Improvement or *complete reversion* to sinus rhythm was observed in the majority of the cases The results were most promising *in* patients with ectopic beats and bigeminy but sinus tachycardia, supraventricular and ventri-

periods in three of the dogs treated with propranolol The heart rates of these animals were well below the minimal rate of forty beats per minute that we have observed in resting, unanaesthetized, well trained dogs

The results obtained in the six dogs treated with 2 mg/kg H 56/28 laevo are also presented in Table 1 One dog showed no response to the drug two showed a partial clearing of the ventricular arrhythmia and three cleared completely within twelve minutes after the drug injection The duration of complete clearing was ten and seventy-four minutes in two of these three animals The third dog still had sinus rhythm when the experiment was terminated forty eight minutes after clearing The incidence of clearing in the H 56/28 laevo group is significantly different from the saline controls ($p=0\ 005$) although the effect was only partial in two of the six animals There is no statistically significant difference between the propranolol and the H 56/28 laevo groups with regard to the incidence of clearing

The remaining group of six dogs received 2 mg/kg of H 56/28 dextro after the development of ventricular tachycardia (Table 1) The arrhythmia persisted in one dog after the injection of the drug whereas the other five immediately reverted to sinus rhythm Complete clearing was sustained for the duration of the experiment (ninety minutes) in these five animals Again, the results obtained in this group differ significantly from those of the control group ($p=0\ 005$) The incidence of clearing is not significantly different between the H 56/28 dextro group and the groups receiving propranolol or H 56 28 laevo The heart rate was consistently lower during the clearing periods after H 56/28 dextro than during the initial accommodation period but the pronounced bradycardia seen in the propranolol treated animals was not observed

Discussion

The accumulative dose of ouabain required to induce ventricular tachycardia in the unanaesthetized dog in the present study is in accordance with the results obtained by other investigators (MOSEY & TYLER 1950, MORROW 1965)

The results have demonstrated the antiarrhythmic activity of the isomers of H 56/28 and confirmed this effect of propranolol against ouabain induced cardiotoxicity (LUCCHESI 1966) The antiarrhythmic effect of 2 0 mg kg of H 56/28 dextro, H 56/28 laevo and propranolol is significantly different from the saline controls The data do not allow for differentiation between the frequency of suppression of ventricular tachycardia by the three drugs H 56 28 dextro seems to have a shorter latency period and a longer duration of action than the other agents It is interesting that the dextro isomer of H 56 28 appears to be at least as potent as the laevo isomer with respect to its anti arrhythmic activity in the present study, whereas its beta adrenergic blocking

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Åblad B M Brogård

Receptor Antagoni

cular tachycardia also responded favorably. The ventricular rate was reduced in cases with atrial fibrillation In a latter series of fourteen patients with a comparable spectrum of arrhythmias LINKO *et al* (personal communication 1967) used the pure dextro isomer of H 56/28 (5 to 20 mg intravenously) but observed no positive effects A comparison of the results in these studies suggests that racemic H 56/28 exerted its effect on the clinical arrhythmias by virtue of the beta adrenergic blocking action of the laevo isomer Beta adrenergic blockade thus appears to be a valuable pharmacological principle in treatment of certain clinical arrhythmias whereas the results obtained in the experimental ouabain-induced arrhythmias in the present study must be attributed to other properties that the two isomers have in common at high dosages

Further studies with the new antiarrhythmic agents are likely to contribute to our understanding of the mechanisms by which rhythmic disturbances are produced Important information may also be obtained with regard to the applicability of results from experimental arrhythmias on clinical questions of pathogenesis and therapeutic effect

Summary

The dextro and laevo isomers of H 56/28 and propranolol can abolish ouabain-induced ventricular tachycardia when injected intravenously in the unanaesthetized dog preparation described

H 56/28 dextro has a shorter onset of antiarrhythmic action with a longer duration than propranolol and H 56/28 laevo

Sinus bradycardia follows suppression of ouabain-induced ventricular tachycardia by both H 56/28 dextro and propranolol, with values lower than those of untreated trained dogs being observed after propranolol

The antiarrhythmic effect of H 56/28 dextro, which is essentially devoid of beta adrenergic receptor blocking activity, suggests that this property is not essential for suppressing ouabain cardiotoxicity

Intrinsic local anaesthetic activity was discussed as a possible mechanism for suppression of ouabain-induced arrhythmia

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Acta pharmacol et toxicol 1967, 25, Suppl 2, 51-62

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The Effect of a ß-Adrenergic Receptor Antagonist (H 56/28) on Lipid Mobilization

By

P. Björntorp, L Ek, S Olsson and G Schröder

Catecholamine-induced increase of serum free fatty acid (FFA) concentration can be inhibited by β-adrenergic receptor antagonists (Pilkington *et al* 1962) This effect is probably due to blockade of the activating action of catecholamines on adipose tissue lipolysis (Björntorp, 1964)

Recently a new β-adrenergic receptor antagonist, 1-(o-allylphenoxy)-3-isopropylamino-2 propanol hydrochloride (H56/28)[1] has been described (Brändström *et al* 1966) In animal pharmacological studies (Åblad *et al* 1967) H 56/28 was shown to be equipotent to propranolol as regards β-receptor blockade in heart as well as vascular and tracheal smooth muscle H 56/28 was further found to possess a weak direct β-receptor stimulating activity on the heart H 56/28 is a racemic mixture of two optical isomers, of which the laevo isomer is mainly responsible for the β-receptor blocking and stimulating actions

In the present study the two optical isomers of H 56/28 were investigated as regards intrinsic effects and influence on the actions of noradrenaline, *in vitro* on adipose tissue metabolism, and *in vivo* on serum FFA concentration in anaesthetized dogs

The effect of a β-adrenergic blocking agent (pronethalol) on the serum FFA concentration during work load in man has previously been reported (Schröder & Björntorp, 1964) It was found that this agent diminished the rise of FFA both during and immediately after work The exact cause of these changes could not be elucidated, however, mainly because of the complex fluxes of FFA during work A further factor complicating the inter-

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Recently a new β adrenergic receptor antagonist, I (o allylphenoxy)-3-isopropylamino-2 propanol hydrochloride (H56/28)[1] has been described (Brandström *et al* 1966) In animal pharmacological studies (Åblad *et al* 1967) H 56/28 was shown to be equipotent to propranolol as regards β-receptor blockade in heart as well as vascular and tracheal smooth muscle H 56/28 was further found to possess a weak direct β-receptor stimulating activity on the heart H 56/28 is a racemic mixture of two optical isomers of which the laevo isomer is mainly responsible for the β receptor blocking and stimulating actions

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pretation of the results was the apparent intrinsic effect of pronethalol not only on lipid mobilization, but also on glucose metabolism

In the present work an investigation was performed on the effect of racemic H 56/28 on lipid mobilization in man during exercise, measuring not only serum FFA concentrations but also different FFA fluxes by means of constant infusion of labelled palmitate

Material and Methods

Investigations on adipose tissue in vitro

The epididymal fat pads from fasting rats were removed and incubated in Krebs-Ringer bicarbonate buffer with 4% albumin as earlier described (BJÖRNTORP & CORRODI, 1966) FFA (DOLE, 1956, FRIEDBERG *et al* 1963) and glycerol (LAMBERT & NEISH, 1950) were determined before and after 120 minutes of incubation

For the evaluation of intrinsic effects the laevo or dextro isomer of H 56/28 was added to the incubation medium in concentrations of 0, or 5×10^{-7} M 5×10^{-6} M 5×10^{-5} M, or 5×10^{-4} M The effects of the H 56/28 isomers were compared to those of norepinephrine in a concentration of 5×10^{-7} M

In a further study the effects of the H 56/28 isomers on norepinephrine-induced lipolysis was investigated by analyzing the response to norepinephrine (5×10^{-7} M) in the presence of one of the isomers in concentrations of 0 or 5×10^{-7}, 5×10^{-6}, 5×10^{-5} or 5×10^{-4} M

The agents used were laevo norepinephrine bitartrate, the laevo isomer of H 56/28 as bitartrate and the dextro isomer as hydrochloride

Statistical significance was assessed by the Student's *t* test

Investigations on anaesthetized dogs

Dogs of mixed breed, weighing 7–10 kg were utilized After 12 hours fasting they were anaesthetized with 30 mg/kg of Nembutal (Abbott) intravenously Catheters were placed in a femoral artery and a femoral vein They were kept open by frequent flushes with 0 9% saline

In each experiment two intravenous infusions of norepinephrine were given with an interval of 90 minutes Each infusion was given by a constant infusion pump for 20 minutes and the dose was 0 45 μg norepinephrine bitartrate per kg and minute *Twenty minutes before the second infusion of norepinephrine* the laevo or dextro isomer of H 56/28 was injected intravenously over two minutes in a dose of 0 2 mg/kg The two isomers of H 56/28 were each studied in two experiments The experimental procedure is essentially that worked out by CARLSON & ORÖ (1962)

The doses of the dextro and laevo isomers of H 56/28 are given as their hydrochloride salts

Arterial blood samples were taken in pre-chilled heparinized tubes for analyses of plasma FFA (DOLE 1956, FRIEDBERG *et al* 1963) and blood glucose enzymatically (LEVIN & LINDE, 1962)

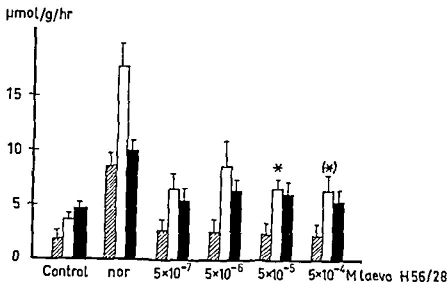
Investigations on patients during exercise

Four patients were examined AB was a woman of 23 years with mild hypertension, EN a man of 54 years with hyperthyroidism, ÅJ a man of 32 years with mild hypertension and FK a man of 45 years, also with mild hypertension

A double blind study was performed Two identical exercise tests were performed on each patient, one after oral treatment with racemic H 56/28 in three daily doses of 40 mg for one week, and the other after corresponding placebo treatment for one week

On the day of investigation the patient, fasted overnight, was given 40 mg H 56/28 or placebo two hours before the start of the experiment A polyethene catheter was placed in the brachial artery and another in a brachial vein after local anaesthesia using 10–15 mg of lidocaine without epinephrine The catheters were kept open by frequent flushes with 0 9% saline About one hour after insertion of the catheters, the patient started to exercise on an electrically braked bicycle ergometer at a work load of 600 kpm/min Blood samples were taken at approximately 5-minute intervals before work and at approximately 2 minute intervals during and after work through the arterial catheter into pre-chilled heparinized tubes Plasma FFA was determined titrimetrically (DOLE, 1956 FRIEDBERG *et al* 1963) In two patients ÅJ and FK, blood glucose concentration was also determined (LEVIN & LINDE 1962) Further an investigation of plasma FFA turnover was carried out in these two patients For this purpose albumin complexed $1-C^{14}$ palmitic acid was given intravenously at a constant infusion rate of about 0 05 μC per minute Infusion was started at least 15 minutes before the first sample was taken After titrimetrical determination of plasma FFA in these two patients, the samples proceeded for measurement of the radioactivity in FFA as described by HAVEL *et al* (1963) Plasma FFA influx and efflux were also calculated as described by HAVEL *et al* (1963) The final heptane phases were evaporated in counting vials and counted in 10 ml scintillation solution (0 4% PPO and 0 01% POPOP in toluene) in a Packard Tri Carb liquid scintillation counter

The albumin complex of $1-C^{14}$ palmitic acid was prepared in the following way The benzene in the ampoule with $1-C^{14}$ palmitic acid (The Radiochemical Centre, Amersham England, CFA 23) was evaporated under nitrogen



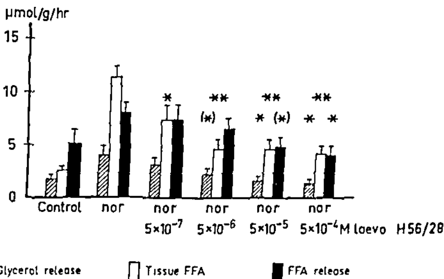
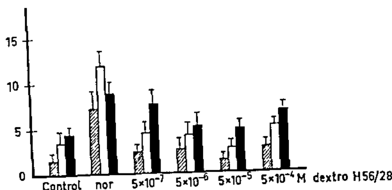
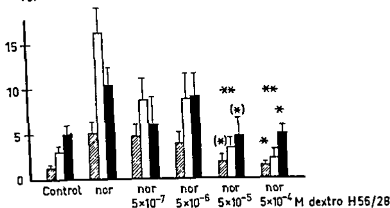


Fig 1 The effects of laevo H 56/28 on basal (upper diagram) and norepinephrine stimulated (lower diagram) metabolism in the rat epididymal fat pad *in vitro* Norepinephrine added as indicated (nor) in a concentration of 5×10^{-7} M Means ± s e m of 4–6 determinations Statistical symbols (*) 0 10>P>0 05, *P<0 05, **P<0 01 Statistical comparison with control levels (upper diagram) and with levels obtained after treatment with norepinephrine alone (lower diagram)

One ml of absolute ethanol and an excess of 0 01 N NaOH for neutralization were added Aliquots of this solution were then evaporated on the steam bath to nearly dryness and then 2 ml of 20% human serum albumin (KABI) were added After careful mixing, this solution was then diluted with 0 9% saline to desired concentration





Glycerol release　Tissue FFA　FFA release

Fig 2 The effects of dextro H 56/28 on basal (upper diagram) and norepinephrine stimulated (lower diagram) metabolism in the rat epididymal fat pad *in vitro* Symbols and denominations as in Fig 1

Results

Investigations on adipose tissue in vitro

Fig 1 shows the effects of laevo H 56/28 on the glycerol and FFA outflow from and the FFA content in the rat epididymal fat pad At a concentration of 5×10^{-5} M and 5×10^{-4} M of laevo H 56/28 a moderate increase of tissue FFA was observed Norepinephrine-induced increase of tissue FFA con-

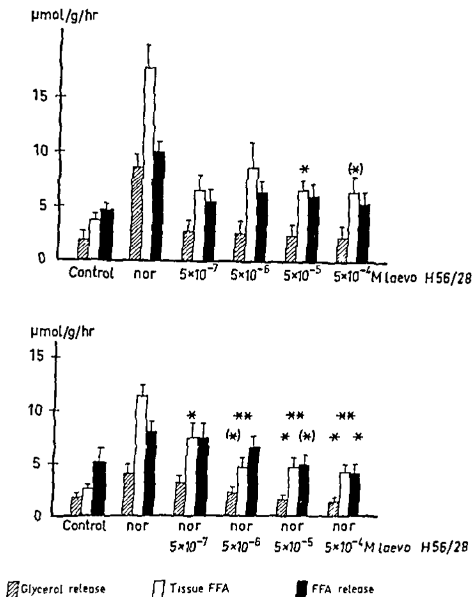


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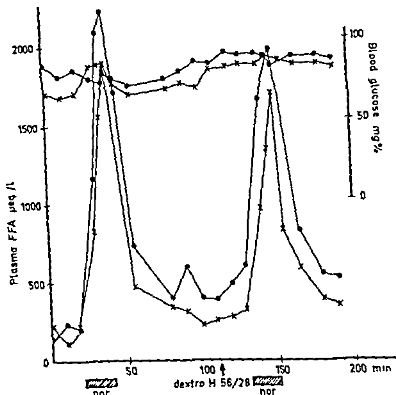


Fig 4 The effects of i v infused norepinephrine (0 45 µg per kg per minute for 20 minutes) on plasma FFA concentration (lower curves) and blood glucose concentration (upper curves) before and after an i v injection of dextro H 56/28 (0 2 mg/kg) in two anaesthetized dogs One dog weighed 8 kg (dots), the other 10 kg (crosses)

The same dose of the dextro form of H 56/28 had no or only a very small effect on basal and norepinephrine stimulated FFA concentrations as seen in Fig 4

No consistent effects on blood glucose were noticed

Investigations on patients during exercise

The first of the two exercise tests was performed in two patients (AB, FK) after H 56/28 treatment (40 mg three times daily) and in the other two patients (EN, ÅJ) after placebo treatment.

The results are given in Fig 5, 6 and 7. At rest, no certain changes in FFA concentration by treatment with racemic H 56/28 was observed in patients AB, ÅJ and FK, while patient EN had a lower FFA concentration than after

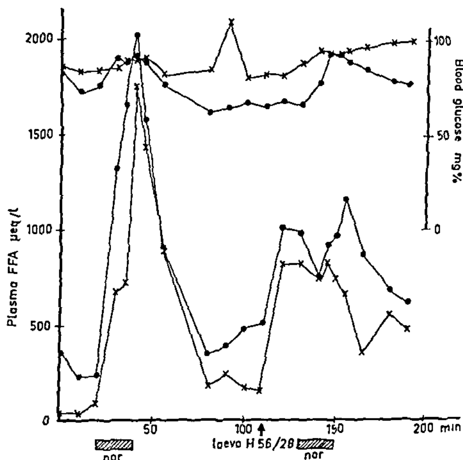


Fig. 3 The effects of i v infused norepinephrine (0 45 µg per kg per minute for 20 minutes) on plasma FFA concentration (lower curves) and blood glucose concentration (upper curves) before and after an i v injection of laevo H 56/28 (0 2 mg/kg) in two anaesthetized dogs One dog weighed 7 kg (dots) the other 9 kg (crosses)

centration was inhibited by laevo *H 56/28* already at a concentration of 5×10^{-7} M At higher concentrations laevo H 56/28 also inhibited norepinephrine induced release of glycerol and FFA into the incubation medium

Fig 2 shows that dextro H 56/28 had no observable intrinsic effect in the *system in question while a significant blockade of norepinephrine induced* effects was first seen at a concentration of 5×10^{-5} M and 5×10^{-4} M

Investigations on anaesthetized dogs

The laevo form of H 56/28 (0 2 mg/kg) caused an increase in plasma FFA when given in a single dose *as seen in Fig 3 Twenty minutes after this* injection, when apparently the intrinsic effect of laevo H 56/28 had levelled off the norepinephrine response was considerably decreased as compared with that before injection

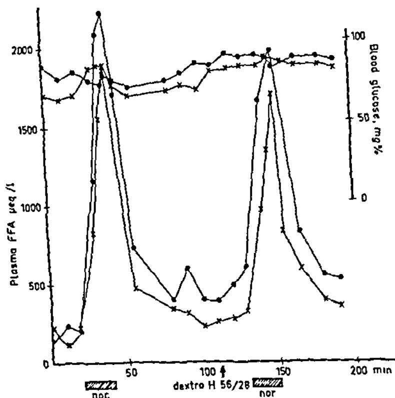


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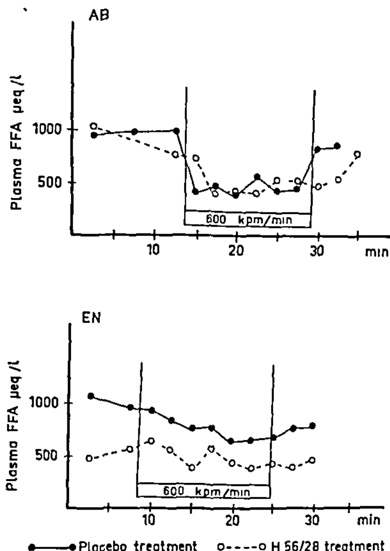


Fig 5 The effects of racemic H 56/28 on plasma concentrations of FFA before during and after exercise in patients AB and EN

placebo treatment Turnover rates for FFA-carbon (HAVEL *et al* 1963) were calculated during rest for patients ÅJ and FK, and found to be 9 2 and 9 4 mM/min after placebo treatment and 8 6 and 10 7 mM/min after H 56/28 treatment In three out of four patients H 56/28 caused a decrease of the FFA concentration during work (ÅJ and FK) and/or a decrease of the FFA peak immediately after exercise (AB and FK) These effects could be explained in patients ÅJ and FK partly by a decrease in FFA influx during and after work, and possibly partly by a small increase of FFA efflux during work

Blood glucose concentration did not seem to change in patients ÅJ and FK either by work or H 56/28 treatment or by H 56/28 treatment plus work

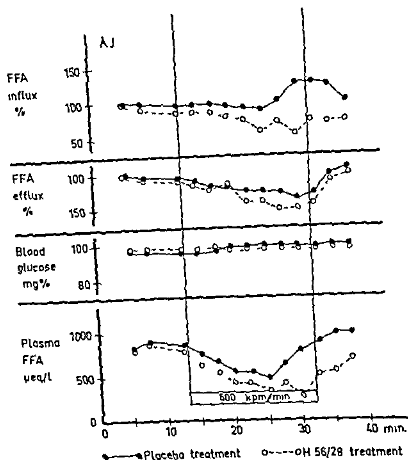


Fig 6 The eff ...
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Discussion

The results of the present *in vitro* studies indicate that H 56/28 antagonized norepinephrine induced lipolysis in adipose tissue, since not only the FFA release but also the increased glycerol outflow (VAUGHAN 1962) was inhibited. This action probably explains why H 56/28 inhibited the increase of serum FFA concentration elicited by norepinephrine in anaesthetized dog ...

... et al (1967) which showed that the cardiovascular β-receptor blocking activity of racemic H 56/28 was predominantly

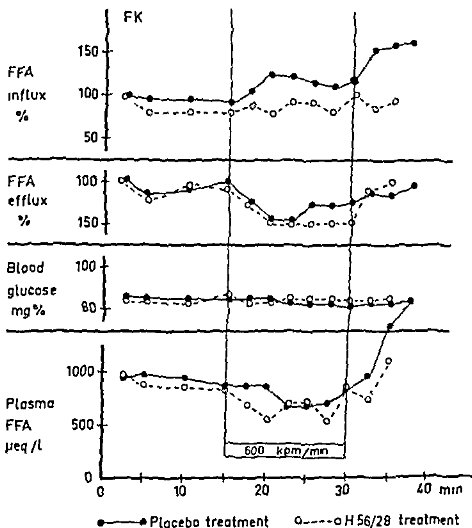


Fig 7 The effect of racemic H 56/28 on plasma FFA concentration, influx and efflux, and blood glucose concentration before, during and after exercise in patient FK

due to its laevo isomer These authors further found that the laevo isomer had a weak β-receptor stimulating action Therefore, it is likely that the slight increase of FFA release produced by laevo H 56/28 in the present investigation is a reflection of a weak intrinsic action on adrenergic receptors in adipose tissue.

Racemic H 56/28 was found to inhibit the increased FFA influx during and immediately after exercise in patients This observation supports earlier suggestions (SCHRÖDER & BJÖRNTORP, 1964) that the increase of FFA influx to the plasma compartment during exercise is at least in part caused by adrenergic activation of lipolysis, presumably in adipose tissue.

The significance of the small increase in FFA efflux seen during work in patients given H 56/28 is difficult to assess, but, if anything, it contributes

to the observed reduction of FFA concentration during H 56/28 treatment

Recently it was found that the racemate of H 56/28 in a dose of 100 mg four times daily decreased plasma FFA significantly in patients with angina pectoris (BJÖRNTORP, 1967) probably also due to a decrease of FFA influx from adipose tissue to plasma

Summary

A β-adrenergic receptor antagonist (H 56/28) was investigated as to its effects on lipid mobilization from adipose tissue On the rat epididymal fat pad *in vitro* the laevo form in high concentrations had an intrinsic norepinephrinelike effect on lipolysis while at low concentrations it inhibited norepinephrine induced increase in lipolysis The dextro form had no measurable intrinsic effect and blocked norepinephrine effects only at high concentrations

The laevo form of the agent increased plasma concentration of free fatty acids in the anaesthetized dog and partly blocked the increase of plasma free fatty acid concentration produced by norepinephrine The dextro form had much less activity

In man the racemate was found to inhibit the increase in plasma free fatty acid concentration during exercise probably due to a blockade of free fatty acid inflow from peripheral stores to plasma

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Acta pharmacol et toxicol 1967 25, Suppl 2, 63-74

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The Effects of Intra-arterially Administered Propranolol[1] and H 56/28[2] on Blood Flow in the Forearm—a Comparative Study of Two ß-adrenergic Receptor Antagonists

By

G JOHNSSON

The two compounds propranolol (BLACK *et al* 1964) and dl-1-(o-allyl-phenoxy)-3-isopropylamino-2-propanol-hydrochloride (H 56/28) (BRÄNDSTRÖM *et al* 1966) have been found to be equipotent *β*-adrenergic receptor antagonists after intravenous administration to both animals (ÅBLAD, BROGÅRD & EK 1967) and man (FORSBERG & JOHNSSON 1967, JOHNSSON, NORRBY & SÖLVELL 1967)

Propranolol is devoid of sympathomimetic properties (BLACK, DUNCAN & SHANKS 1965, ÅBLAD BROGÅRD & EK 1967) H 56/28, however, in addition to its *β*-receptor blocking action also has a slight *β*-receptor stimulating activity (ÅBLAD, BROGÅRD & EK 1967) and this latter action seems to modify the effect of the drug on cardiac function in man under basal conditions (FORSBERG & JOHNSSON 1967)

In the present study an attempt was made to compare the vascular actions of propranolol and H 56/28 in the human forearm The drugs were administered into the brachial artery and their effects on basal blood flow and on isoprenaline induced vasodilatation were investigated Two doses of the *β*-adrenergic blocking agents (0 05 and 0 5 mg) were administered intra-arterially The lower dose was intended to approximate the tissue concentration achieved after the administration of doses commonly used clinically

The racemic forms of propranolol and H 56/28 were generally used in the study but some experiments with the dextro isomer of H 56/28 were also

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[2] Aptin® Hässle, Sweden

performed It is known that the β-receptor blockade of the agents and in the case of H 56/28 also its β-receptor stimulating action is *predominantly* exerted by their levo isomers (HOWE & SHANKS 1966, ÅBLAD, BROGÅRD & EK 1967)

Methods

Thirty-one experiments were performed on healthy male students according to a procedure modified from that of ÅBLAD, JOHNSSON & HENNING (1961) The brachial artery of one forearm (test forearm) was anesthetized in the cubital fold with about 1 ml 1 per cent lidocain A teflon catheter (outer diameter 0 6 mm) was then introduced into the artery via a Cournand artery cannula The catheter was advanced proximally for about 8 cm into the artery, so that the tip of the catheter had about the same position in the artery in the different experiments The catheter was connected to a motor-driven syringe delivering isotonic saline or test drugs dissolved in isotonic saline at a constant rate (0 6 ml/minute)

A plethysmograph was applied to the upper part of each forearm and placed at the level of the sternal angle The plethysmographs were of the type described by FOLKOW, GRIMBY & THULESIUS (1958), but the expansion chamber was modified according to DAHN (1964) The forearms were covered with sleeves of thin latex The temperature of the plethysmograph water was 34°C and the room temperature 22±0 5°C Cuffs were applied round the arm just proximal and distal to the pletysmographs The blood flow was measured simultaneously in both forearms by rapidly inflating the proximal cuffs once or twice a minute to a pressure of 50 mm Hg The duration of each inflation was about 10 seconds The volume increase in the forearms was recorded by ink-writing piston-recorders The distal cuffs were inflated to 230 mm Hg at least one minute before measurement of the blood flow The hand circulation was blocked during the administration of the test drugs

The results show that the blood flow was approximately the same in the two forearms before administration of the drugs and there was no indication that the catherization of the brachial artery interfered with the blood flow to the forearm

Two main types of experimental procedures were performed In one series of experiments only the effects of the β-adrenergic blocking agents on basal forearm blood flow were studied At first the basal blood flow was recorded for about 30 minutes and after that a β-adrenergic blocking agent was infused into the artery for 4 minutes in a total dose of 0 05 mg Fifty minutes later the same drug was given for 4 minutes in a total dose of 0 5 mg, and the recording of the blood flow proceeded for another 20 minutes The effects of propranolol and H 56/28 on the forearm blood flow were tested on each

of four subjects and the interval between the two studies on the same subject was always more than 5 days The dextro isomer of H 56/28 was tested on 3 subjects

In another type of experiments the interference of propranolol, H 56/28 or the dextro form of H 56/28 with the vasodilator effect of isoprenaline in the forearm was studied After recording the blood flow for 20–30 minutes isoprenaline (0 015 μg/minute l-isoprenaline sulphate except on one subject who received 0 022 μg/minute) was infused intra-arterially for five minutes Thirty minutes later the β-adrenergic blocking agent was given intra-arterially during 4 minutes in a total dose of 0 05 mg and 10 minutes after that the test with isoprenaline was repeated After another 35 minutes the same test procedure with the β-adrenergic blocking agent, now in a ten fold dose, and isoprenaline was repeated From these experiments also the initial effects of the β-adrenergic blocking agents on the basal blood flow can be estimated The same type of experiment was also performed with a placebo solution *instead of* a β-adrenergic blocking agent This type of experiment with propranolol, H 56/28 and placebo was performed on each of five subjects and the interval between two studies on the same subject was always more than 5 days The dextro isomer of H 56/28 was studied on one subject in this type of experiment

In order to establish the dose-effect relationship on the forearm blood flow between intra arterially and intravenously administered β-adrenergic blocking agents some additional studies were made The experimental design was the same as in the above study but propranolol and H 56/28 were infused *intravenously* instead of intra-arterially in a dose of 2 mg per minute during 5 minutes, while isoprenaline was given intra arterially as in the previous studies

The drugs used were racemic propranolol hydrochloride (propranolol), [illegible] (H 56/28), [illegible] The infused [illegible] solutions contained as a preservative 0 001 per cent ascorbic acid All the doses refer to the salts of the drugs.

The *basal values* of blood flow in the results represent the mean values of 5–8 measurements on each subject during each 5-minute period The effects of *isoprenaline* are represented by the mean values of 4–5 measurements on each subject during the last 2 minutes of the infusion when the effect reached a steady state level

Results are reported as means±standard errors of the means The statistical analysis was based on the *t*-test (FISHER 1958)

Results

A *Interference of racemic propranolol, racemic H 56/28, the dextro form of H 56/28 and placebo with the vasodilator effect of isoprenaline*

The interference of *intra arterially* infused propranolol, H 56/28 and placebo with the vasodilator effect of isoprenaline administered in the same manner were all studied on the same 5 subjects (table 1, fig 1) Before the infusion of a β adrenergic blocking agent isoprenaline increased the blood flow of the ipsilateral forearm (test forearm) in the three series of experiments by about 4 ml per 100 ml tissue per minute (in the following this is abbreviated as "ml") When propranolol or H 56/28 was infused in a small dose, 0·05 mg, the blood flow response to isoprenaline was reduced by about 75 per cent Propranolol or H 56/28 in a higher dose, 0·5 mg intra-arterially, blocked the effect of isoprenaline almost completely The effect of 0·5 mg H 56/28 in the dextro form was about the same as that of 0·05 mg propranolol or H 56/28 (table 1) Placebo had no effect on the blood flow increase after isoprenaline The blood flow of the other forearm (control forearm) remained on the whole unchanged throughout the experiments

Administration of propranolol *intravenously* in a dose of 10 mg reduced the effect of intra-arterially administered isoprenaline by 83 and 90 per cent on the forearm blood flow The corresponding values in two experiments with the same dose of H 56/28 were 84 and 86 per cent The arterial blood pressure was not changed after intravenous administration of the two β-adrenergic blocking agents The β adrenergic blocking effect of the two agents on the forearm blood flow was thus somewhat more pronounced when they were administered intravenously in a dose of 10 mg than after 0·05 mg intra arterially

B *Effects of intra arterially administered racemic propranolol, racemic H 56/28 and the dextro isomer of H 56/28 on the basal blood flow in the forearm*

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In nine experiments the blood flow changes in the forearm after administration of propranolol were studied (table 2) After the infusion of the drug into a brachial artery in a *low dose, 0·05 mg*, there was a small decrease of the blood flow in this forearm (test forearm) Thus 7–12 minutes after the start of the infusion the blood flow decrease in the test forearm was maximal 0·4±0·20 ml compared to the 5-minute period immediately before the drug Very small, variable changes occurred in the blood flow of the other forearm (control forearm) The change was 0·0±0·18 ml in this 7–12 minutes after the start of the infusion The relation between test and control forearm blood flow after propranolol infusion was thus different from the relation before

Table 1 *Increase* of blood *flow in* the forearms produced by *isoprenaline* before and after *infusion* of β adrenergic block*ing* drugs and placebo The *agents were* infused into the brach*ial artery* of the test forearm Blood flow *increase* in ml per *100 ml tissue* per minute Mean ± s e *m*

Drug	Effect of *isoprenaline* (increase of blood flow)								
	Before β *blocking* drug or placebo			After β blocking drug or placebo					
				0 05 mg			0 5 mg		
	test forearm	control forearm	difference test-control	test forearm	control forearm	difference test-control	test forearm	control forearm	difference test control
Propranolol *n*=5	4 3±0 35	0 1±0 10	4 2±0 40	1 1±0 49	0 0±0 13	1 1±0 37	0 4±0 07	0 2±0 12	0 2±0 13
H 56/28 *n*=5	4 2±0 48	0 1±0 10	4 1±0 50	1 1±0 25	0 0±0 22	1 1±0 24	0 1±0 11	0 1±0 09	0 0±0 03
H 56/28 dextro *n*=1	4 2	0 1	4 1	2 9	0	2 9	1 3	0	1 3
Placebo *n*=5	3 9±0 76	−0 3±0 27	4 2±0 81	4 1±0 62	−0 2±0 12	4 3±0 69	4 5±0 75	0 0±0 18	4 5±0 73

Results

A *Interference of racemic propranolol, racemic H 56/28, the dextro form of H 56/28 and placebo with the vasodilator effect of isoprenaline*

The interference of *intra arterially* infused propranolol, H 56/28 and placebo with the vasodilator effect of isoprenaline administered in the same manner were all studied on the same 5 subjects (table I, fig 1) Before the infusion of a β-adrenergic blocking agent isoprenaline increased the blood flow of the ipsilateral forearm (test forearm) in the three series of experiments by about 4 ml per 100 ml tissue per minute (in the following this is abbreviated as "ml") When propranolol or H 56/28 was infused in a small dose, 0 05 mg, the blood flow response to isoprenaline was reduced by about 75 per cent Propranolol or H 56/28 in a higher dose, 0 5 mg intra-arterially, blocked the effect of isoprenaline almost completely The effect of 0 5 mg H 56/28 in the dextro form was about the same as that of 0 05 mg propranolol or H 56/28 (table 1) Placebo had no effect on the blood flow increase after isoprenaline The blood flow of the other forearm (control forearm) remained on the whole unchanged throughout the experiments

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B *Effects of intra-arterially administered racemic propranolol, racemic H 56 28 and the dextro isomer of H 56/28 on the basal blood flow in the forearm*

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Table 1 *Increase* of blood flow in the forearms produced by isoprenaline before and after infusion of β adrenergic blocking drugs and *placebo* The agents were infused into the brachial artery of the test forearm Blood flow *increase in ml per 100 ml* tissue per minute Mean ± s e m

Drug	Effect of isoprenaline (increase of blood flow)								
	Before β blocking drug or placebo			After β blocking drug or placebo					
				0 05 mg			0 5 *mg*		
	test forearm	control forearm	difference test control	test forearm	control forearm	difference test-control	test forearm	control forearm	difference *test control*
Propranolol *n*=5	4 3±0 35	0 1±0 10	4 2±0 40	1 1±0 49	0 0±0 13	1 1±0 37	0 4±0 07	0 2±0 12	0 2±0 13
H 56/28 *n*=5	4 2±0 48	0 1±0 10	4 1±0 50	1 1±0 25	0 0±0 22	1 1±0 24	0 1±0 11	0 1±0 09	0 0±0 03
H 56/28 dextro *n*=1	4 2	0 1	4 1	2 9	0	2 9	1 3	0	1 3
Placebo *n*=5	3 9±0 76	−0 3±0 27	4 2±0 81	4 1±0 62	−0 2±0 12	4 3±0 69	4 5±0 75	0 0±0 18	4 5±0 73

Results

A *Interference of racemic propranolol, racemic H 56/28, the dextro form of H 56/28 and placebo with the vasodilator effect of isoprenaline*

The interference of *intra-arterially* infused propranolol, H 56/28 and placebo with the vasodilator effect of isoprenaline administered in the same manner were all studied on the same 5 subjects (table 1, fig 1) Before the infusion of a β-adrenergic blocking agent *isoprenaline increased the blood flow* of the ipsilateral forearm (test forearm) in the three series of experiments by about 4 ml per 100 ml tissue per minute (in the following this is abbreviated as "ml") When propranolol or H 56/28 was infused in a small dose, 0 05 mg, the blood flow response to isoprenaline was reduced by about 75 per cent Propranolol or H 56/28 in a higher dose, 0 5 mg intra-arterially, blocked the effect of isoprenaline almost completely The effect of 0 5 mg H 56/28 in the dextro form was about the same as that of 0 05 mg propranolol or H 56/28 (table 1) Placebo had no effect on the blood flow increase after isoprenaline The blood flow of the other forearm (control forearm) remained on the whole unchanged throughout the experiments

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In nine experiments the blood flow changes in the forearm after administration of propranolol were studied (table 2) After the infusion of the drug into a brachial artery in a *low dose, 0 05 mg*, there was a small decrease of the blood flow in this forearm (test forearm) Thus 7-12 minutes after the start of the infusion the blood flow decrease in the test forearm was maximal $0\,4 \pm 0\,20$ ml compared to the 5-minute period immediately before the drug Very small, variable changes occurred in the blood flow of the other forearm (control forearm) The change was $0\,0 \pm 0\,18$ ml in this 7-12 *minutes* after the start of the infusion The relation between test and control forearm blood flow after propranolol infusion was thus different from the relation before

Table 2. Effects of intra arterially *infused* β adrenergic blocking agents on the blood flow in the *forearms* The drugs were infused into the brachial artery of the test forearm for four minutes Blood flow in ml per 100 ml tissue per minute Mean ±s e m

Drug	Dose	Blood flow before infusion of β-blocking drug		Blood flow after start of infusion of β blocking drug					
				1 6 min		*7 12 min*		*13-18 min*	
		test forearm	control forearm	test forearm	control forearm	test forearm	control forearm	test forearm	control forearm
Propranolol *n*=9	0 05 mg	3 6±0 41	3 3±0 29	3 5±0 40	3 4±0 31	3 2±0 41	3 3±0 30	3 3±0 42	3 4±0 36
Propranolol *n*=9	0 50 mg	3 1±0 35	3 3±0 33	4 0±0 37	3 5±0 26	3 0±0 28	3 0±0 24	3 0±0 31	3 0±0 24
H 56/28 *n*=9	0 05 mg	3 4±0 34	3 4±0 38	3 6±0 39	3 4±0 38	3 5±0 38	3 4±0 38	3 6±0 43	3 4±0 38
H 56/28 *n*=9	0 50 mg	3 5±0 33	3 3±0 33	4 4±0 37	3 3±0 28	3 2±0 33	3 0±0 30	3 3±0 25	3 0±0 26

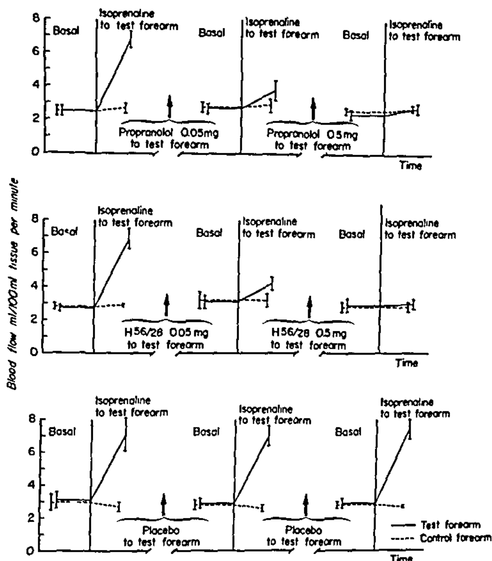


Fig 1 Effects of intra-arterial infusions of isoprenaline into the brachial artery of the test forearm side (solid line) on blood flow in the forearm before and after administration of β-adrenergic blocking dracgs and placebo into the same artery The blood flow of the other forearm served as control (dashed line) Mean ± s e m (n 5)

propranolol was infused The "*net effect*" of propranolol can be calculated according to the formula (I)

"*Net effect*" = (*test minus control forearm blood flow after the drug*) *minus* (*test minus control forearm blood flow before the drug*),

with this mode of calculation a "net decrease" of 0 4±0 15 ml ($p<0\ 05$) was found in the test forearm 7–12 minutes after the start of the infusion of propranolol This decrease then persisted essentially unchanged throughout the studies, which proceeded for 50 minutes on 4 subjects

Table 2. Effects of intra arterially infused β adrenergic blocking agents on the blood flow in the forearms The drugs were infused into the brachial artery of the test forearm for four minutes Blood flow in ml per 100 ml tissue per minute Mean ±s e m

Drug	Dose	Blood flow before infusion of β blocking drug		Blood flow after start of infusion of β blocking drug: 1 6 min		7 12 min		13 18 min	
		test forearm	control forearm	test forearm	control forearm	test forearm	control forearm	test forearm	control forearm
Propranolol n=9	0 05 mg	3 6±0 41	3 3±0 29	3 5±0 40	3 4±0 31	3 2±0 41	3 3±0 30	3 3±0 42	3 4±0 36
Propranolol n=9	0 50 mg	3 1±0 35	3 3±0 33	4 0±0 37	3 5±0 26	3 0±0 28	3 0±0 24	3 0±0 31	3 0±0 24
H 56/28 n=9	0 05 mg	3 4±0 34	3 4±0 38	3 6±0 39	3 4±0 38	3 5±0 38	3 4±0 38	3 6±0 43	3 4±0 38
H 56/28 n=9	0 50 mg	3 5±0 33	3 3±0 33	4 4±0 37	3 3±0 28	3 2±0 33	3 0±0 30	3 3±0 25	3 0±0 26

When propranolol was infused in a *higher dose 0 50 mg* (accumulated dose 0 55 mg) there was a transient blood flow increase in the test forearm immediately after the start of the infusion (table 2) The blood flow increase in the test forearm was *0 9±0 15 ml* ($p<0\ 001$) *during the period 1–6 minutes* after the start of the infusion, during the next 5-minute period the blood flow in the test forearm was again of the same order of magnitude as before this dose of the drug Small, insignificant changes occurred in the blood flow of the control forearm The "net effect" was an increase of 0 7±0 11 ml ($p<0\ 001$) in the test forearm 1–6 minutes after the start of the infusion and 0 2±0 10 ml during the next 5-minute period

II *Effects of racemic H 56/28*

The effects of intra-arterially administered H 56/28 on the blood flow in the forearm were studied on the same 9 subjects as employed in the propranolol study (table 2) *After the infusion of the drug in a low dose 0 05 mg*, there was no significant change of the blood flow in the test forearm Thus 7–12 minutes after the start of the infusion the blood flow increase in the test forearm was 0 1±0 14 ml compared to the 5-minute period immediately before the drug The corresponding change of the blood flow in the control forearm was 0 0±0 19 ml If the "net effect" was calculated according to formula I, there was an insignificant increase of the blood flow in the test forearm amounting to 0 1±0 15 ml 7–12 minutes after the start of the infusion This change persisted essentially unchanged throughout the studies, which proceeded for 50 minutes on 4 subjects

H 56/28 when infused in a *higher dose, 0 50 mg*, (accumulated dose 0 55 mg) increased the blood flow of the test forearm immediately after the start of the infusion to the same degree as propranolol (table 2) The blood flow increase in this forearm was significant (0 9±0 27 ml $p=0\ 01$) only during *the period 1–6 minutes after the start of the infusion* The blood flow of the control forearm showed insignificant changes and the "net effect" was plus 0 9±0 20 ml ($p<0\ 01$) 1–6 minutes and 0 0±0 09 ml 7–12 minutes after the start of the intra-arterial infusion of the drug

In an additional experiment propranolol (0 5 mg) was infused intra-arterially 10 minutes before the administration of the same dose of H 56 28 Also now H 56/28 induced a blood flow increase and this amounted to 0 8 ml (30 per cent)

III *Effects of the dextro isomer of H 56/28*

In three experiments the forearm blood flow after the dextro form of H 56/28 was studied *During the infusion of the drug into a brachial artery in a total dose of 0 50 mg* there was a transient increase of the blood flow in the test forearm of the same magnitude and duration as when 0 50 mg propranolol

or H 56/28 were given The two latter drugs increased the blood flow in the test forearm by on an average 25±3 4 and 28±7 3 per cent respectively 1–6 minutes after the start of the infusion The corresponding mean value for the three experiments with the dextro isomer of H 56/28 was 23±4 9 per cent

Discussion

The vasodilator action of isoprenaline is by Ahlquist s definition due to β adrenergic receptor activation (AHLQUIST 1948) In the present study intra arterially administered propranolol and H 56/28 were found to be equipotent antagonists of isoprenaline induced vasodilatation in the forearm while H 56/28 dextro was considerably less active than the racemate These findings are in good agreement with results from animal and human pharmacological studies with intravenously administered propranolol and H 56/28 which indicate that the two agents have equal cardiovascular β receptor blocking activity (ÅBLAD BROGÅRD & EK 1967 FORSBERG & JOHNSSON 1967 JOHNSSON NORRBY & SÖLVELL 1967) and that this action is predominantly exerted by their levo isomers (HOWE & SHANKS 1966 ÅBLAD BROGARD & EK 1967, ÅBLAD *et al* 1967)

The clinically used intravenous doses of propranolol and H 56/28 usually vary between 1 10 mg i e about 0 015-0 15 mg per kg body weight In the present study the vasodilator response in the forearm to intra arterially administered isoprenaline was inhibited somewhat more by an intravenous dose of 10 mg propranolol or H 56/28 than by an intra arterial dose of 0 05 mg This result indicates that the lower intra arterial dose of propranolol or H 56/28 used in the present experiments gave a forearm tissue concentration within the range attained after clinically used intravenous doses This range was probably exceeded after the higher intra arterial dose of the drugs (0 5 mg)

The *lower* intra arterial dose of propranolol (0 05 mg) produced a small but significant decrease of basal blood flow in the forearm This effect appeared during the infusion of the drug and persisted at a relatively stable level from 5 to 45 minutes after the termination of the drug administration A similar time effect relationship has been found to characterize the propranolol induced blockade of the isoprenaline induced vasodilator response in the forearm (JOHNSSON unpublished results) Therefore it seems likely that the vaso constrictor response of propranolol was due to inhibition of an endogenous β-receptor activation possibly elicited by small amounts of circulating adrena line NAKANO & KUSAKARI (1965 1966) in dogs also recorded a blood flow decrease after propranolol administered intra arterially while BRICK et al (1966) did not record any changes in the forearm blood flo[illegible] ision of pron[illegible]

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The same intra arterial dose of H 56/28 (0 05 mg) probably inhibited the

vasodilator action of endogenous catecholamines since this drug antagonized the isoprenaline response to the same degree as propranolol However, no decrease of basal blood flow was seen after H 56/28 This circumstance may indicate that H 56/28 has a weak β-receptor stimulating action in man not only in heart (FORSBERG & JOHNSSON, 1967), but also on blood vessels Such an action might have produced a weak vasodilatation which counter-balanced the vasoconstrictor effect due to β-receptor blockade of endogenous catecholamines

This interpretation is supported by recent studies on reserpinized dogs (EK & JOHNSSON, 1967) Thus after administration of H 56/28 (dose 0 02 mg) *into the femoral artery the blood flow increased somewhat in the corresponding* leg This effect of H 56/28 appeared to have the same time-effect relationship as its β-receptor blocking activity Furthermore the blood flow increase after H 56/28 could be blocked by propranolol No systemic effects of H 56/28 administered in this way could be observed Propranolol, when administered into the femoral artery of reserpinized dogs in a dose of 0 02 mg, did not change the blood flow in the leg Thus these results indicate that H 56/28 has a weak β-receptor stimulating effect resulting in a vasodilatation of the skeletal muscle vessels, while propranolol is devoid of such a property

In the *higher* intra-arterial dose (0 50 mg) both propranolol and H 56/28 elicited a transient vasodilatation of equal magnitude Such a transitory vasodilatory effect of propranolol has previously been demonstrated in man (SCHOOP & SCHMIDTKE 1965) and dog (NAKANO & KUSAKARI 1965, 1966, SHANKS 1967) after injection of the drug intra-arterially These investigators concluded that this vasodilator action of propranolol was not due to β-receptor stimulation since it was not influenced by previous injections of propranolol or dichloroisoprenaline This conclusion seems to be relevant also for H 56/28 The dextro isomer of H 56/28 is virtually devoid of β-receptor stimulating action (ÅBLAD, BROGÅRD & EK 1967) and yet it elicited a transient vasodilatation equal to that of the racemate Furthermore it was not possible to block this acute vasodilator effect of H 56/28 with propranolol in a dose which completely abolished the effect of isoprenaline

The mechanism of this "direct" (not mediated via the β-receptor) vasodilator action of the agents is not known SHANKS (1967) suggested that the transient vasodilator effect of propranolol was related to its local anesthetic action The present results are in agreement with such an interpretation as propranolol, H 56/28 and also its dextro isomer have been shown to possess local anesthetic activity in the same range as lidocaine (ÅKERMAN 1967—personal communication) Local anesthetic agents are known to have vasodilator properties, due to a depression of vascular smooth muscle tone (TRUANT & TAKMAN 1965)

After intravenous administration of propranolol and H 56/28 in relatively

high doses (10 mg) in man no decrease of the arterial mean blood pressure or peripheral resistance could be observed corresponding to the initial "direct" vasodilator effect of the drugs (FORSBERG & JOHNSSON 1967) Therefore it seems probable that the "direct" effect of the drugs on vessels is not of importance when the drugs are utilized therapeutically in the dose mentioned

Summary

The β-adrenergic blocking agents, racemic propranolol and 1-(o-allyl-phenoxy)-3-isopropyl-amino-2-propanol hydrochloride (H 56/28) in racemic and dextro form were compared with respect to their effects on the basal forearm blood flow and their interference with repeated infusions of isoprenaline in man The β adrenergic blocking drugs were administered unilaterally into a brachial artery (test forearm) The doses used were calculated to yield tissue concentrations of the drug in the arm either within (0 05 mg) or above (0 5 mg) the range associated with systemic administration of therapeutic doses

Racemic propranolol and H 56/28 decreased the effect of isoprenaline administered intra-arterially into the test forearm to the same extent The lower dose of the two drugs reduced the vasodilator effect of *isoprenaline* by about 75 per cent, while the higher dose of the two drugs abolished it almost completely The dextro isomer of H 56/28 was a much weaker β-adrenergic blocking substance than racemic propranolol and H 56/28

When administered in the *lower* dose, racemic propranolol decreased the *basal blood flow* in the test forearm somewhat, while the flow was essentially unchanged after the racemic form of H 56/28 This effect of propranolol may be due to its β-adrenergic blocking effect, while racemic H 56/28 has a weak stimulating effect of the β-adrenergic receptors, which may counter-balance its β-receptor blocking activity During infusion of the *higher* dose of the three β-adrenergic blocking agents there was initially a significant increase of the blood flow in the test forearm, which disappeared almost completely after the termination of the infusion This acute vasodilator effect of the three drugs is not related to their actions on the β-adrenergic receptor A possible explanation for this initial vasodilator effect of the drugs isdiscussed

Acknowledgements

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Hemodynamic Effects of Propranolol[1] and H 56/28[2] in Man — a Comparative Study of two β-adrenergic Receptor Antagonists

By

S Å FORSBERG and G JOHNSSON

Recently a new β receptor antagonist—H 56/28 (dl-1 (o-allylphenoxy)-isopropylamino-2 propanol-hydrochloride) has been described (BRÄNDSTRÖM *et al* 1966) and its pharmacological properties have been compared to those of propranolol (ÅBLAD, BROGÅRD & EK 1967) In animals H 56/28 and propranolol were found to be equipotent β-receptor antagonists and they had the same duration of action after intravenous administration However, the two agents affected the basal cardiac function differently While propranolol reduced cardiac rate, contractile force and cardiac output, equipotent β-receptor blocking doses of H 56/28 did not alter these variables This difference could be ascribed to the fact that H 56/28 also possesses a slight direct β-receptor stimulating activity (ÅBLAD, BROGÅRD & EK 1967), while propranolol is devoid of such properties (BLACK, DUNCAN & SHANKS 1965)

The effects of H 56/28 and propranolol also have been compared on healthy volunteers The changes in heart rate and auscultatory blood pressure recordings at basal conditions and during intravenous isoprenaline infusions yielded results that were in essential agreement with those obtained in the animal studies referred to above (ÅBLAD *et al* 1967, JOHNSSON, NORRBY & SÖLVELL 1967)

In the present investigation an attempt was made to compare the hemodynamic effects of H 56/28 and propranolol in healthy volunteers by more direct methods The influence of either H 56/28 or propranolol on cardiac output as well as on the blood pressure in a brachial artery and the right atrium was analyzed In addition the β-receptor blocking activities of the two agents were compared by studying their influence on isoprenaline-induced changes of heart rate and intra-arterial blood pressure A preliminary report of this study has been presented previously (FORSBERG & JOHNSSON 1967)

[1] Inderal®, ICI England
[2] Aptin®, Hässle Sweden

Methods

The experiments were performed on 5 healthy male students, 20–25 years of age. Each volunteer participated in both the propranolol and H 56/28 studies. The interval between the two experiments in each subject was at least 5 days. Two of the subjects received propranolol as the first treatment, the remaining three subjects received H 56/28 as the first drug. The studies commenced in the morning after the subjects had fasted for 12 hours. They were recumbent and catheters were placed percutaneously into a brachial artery, into the right atrium and into a cubital vein. Pressures were measured by means of variable inductance transducers (Elema-Schonander AB) and were recorded together with an electrocardiogram photographically with the aid of a multichannel recorder. Cardiac output was determined by the indicator dilution technique, using sulfobromophtalein sodium as an indicator (WASSÉN 1956, FORSBERG 1964).

The total peripheral vascular resistance (in arbitrary PR units) was calculated as follows:

$$\frac{\text{mean arterial blood pressure (mm Hg)} - \text{mean right atrial blood pressure (mm Hg)}}{\text{cardiac output (l/min.)}}$$

The experiments proceeded according to the following protocol. The study commenced with continuous recordings of arterial blood pressure, right atrial mean blood pressure and ECG. Ten minutes after a circulatory steady state level was reached l-isoprenaline sulphate was infused during 5 minutes in a dose of 0.03 µg per kg body weight per minute. Thirty minutes after the termination of the infusion of isoprenaline the cardiac output was determined and 15 minutes later the infusion of the β-blocking agent was started. The racemic forms of either propranolol or H 56/28 were given intravenously during 5 minutes in a dose of 2 mg/minute so that the total dose was 10 mg. Determinations of cardiac output were then made 3 and 40 minutes after the start of the infusion of the β-blocking agent. Finally, 45 minutes after the administration of the β-blocking agent, isoprenaline was infused again, now in a dose of (0.09 µg/kg body weight per minute). The reason for administering a higher dose of isoprenaline after the β-blocking drugs was that preliminary studies had shown that the effects of the previous lower dose of isoprenaline were almost completely blocked by 10 mg intravenously of propranolol or H 56/28 (cf. JOHNSSON, NORRBY & SÖLVELL 1967). A total blockade makes a quantitative comparison between the two β-blocking agents impossible.

The results are reported as means ± standard errors of the means. Statistical analysis was based on the *t*-test (FISHER 1958).

Results

A *Interference of propranolol and H 56/28 with the cardiovascular effects of isoprenaline*

Table 1 shows that propranolol and H 56/28 were equally active antagonists to isoprenaline-induced changes of heart rate, arterial pulse pressure and diastolic blood pressure

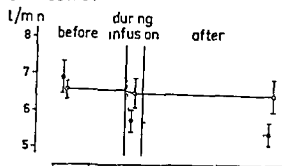
B *Hemodynamic effects of propranolol and H 56/28*

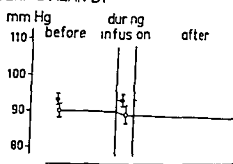
Fig 1 summarizes the recorded hemodynamic effects of propranolol and H 56/28 on cardiac output, heart rate, stroke volume, mean arterial blood pressure, total peripheral vascular resistance and mean right atrial blood pressure

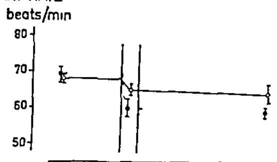
After *propranolol* the cardiac output decreased in all subjects This effect was evident already three minutes after the start of the administration Thirty-five minutes after the administration of propranolol cardiac output was 1 6±0 37 l/min ($p<0\,02$) below the pre drug level At the same time the decrease of the heart rate was 10±2 6 beats/min ($p<0\,02$) In four of the five subjects the stroke volume decreased while there was an increase in the fifth subject The mean change of the stroke volume 35 minutes after the administration of propranolol was minus 10±8 0 ml At the same time the

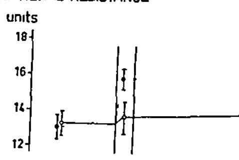
Table 1 Effects of isoprenaline on heart rate, arterial pulse pressure and diastolic blood pressure before and after propranolol and H 56/28 (10 mg i v) The effects of isoprenaline on these parameters were calculated as the difference between the mean of the values obtained during the last two minutes of the infusion of isoprenaline and the mean of the basal values obtained during the last ten minutes before the infusion Mean±s e m (n 5)

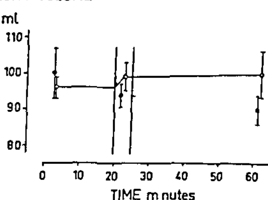
Drug	Before β-blocking agent			45 minutes after β-blocking agent		
	Isoprenaline 0 03 μg/kg b w /min			Isoprenaline 0 09 μg/kg b w /min		
	Increase of heart rate beats/min	Increase of pulse pressure mm Hg	Decrease of diastolic pressure mm Hg	Increase of heart rate beats/min	Increase of pulse pressure mm Hg	Decrease of diastolic pressure mm Hg
Propranolol racemate 10 mg i v	36±5 5	36±4 4	17±2 4	13±1 4	23±2 4	6±1 1
H 56 28 racemate 10 mg i v	38±2 7	35±7 0	15±3 9	11±1 3	26±3 1	3±0 6

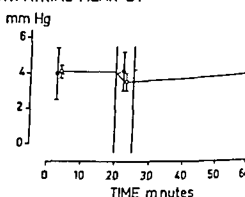












•—•PROPRANOLOL ○——○H 56/28

Fig 1 Effects of propranolol and H 56/28 (10 mg i v of the racemic forms) on cardiac output heart rate stroke volume mean arterial blood pressure total peripheral vascular resistance and mean right atrial blood pressure Mean±s e m ($n=5$)

mean arterial blood pressure tended to decrease slightly but this change was not statistically significant (decrease 3±1 2 mm Hg compared to the pre drug value) Consequently there was an increase of the calculated total peripheral vascular resistance (3 0±0 8 P R units $p<0\ 02$) In four cases of five the mean right atrial blood pressure had increased 35 minutes after propranolol while it was unchanged on the fifth subject (mean increase 1 0±0 6 mm Hg)

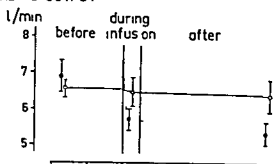
After *H 56/28* the mentioned variables were not significantly altered Thirty-five minutes after the infusion of the drug the mean cardiac output was practically the same as the pre drug value (−0 1±0 33 l/min) Insignificant changes had occurred with the heart rate (−4±2 8 beats/min) and stroke volume (+4±4 8 ml) The mean arterial blood pressure and the mean right atrial blood pressure were only slightly influenced by H 56/28 35 minutes after the administration of the drug (decrease 2±2 1 and 0 1±0 3 mm Hg respectively from the pre drug value)

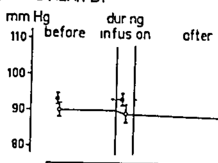
The *continuous effects* of propranolol and H 56/28 on the basal heart rate and arterial blood pressures are shown in fig 2 The arterial blood pressures are presented as the pulse pressure and the diastolic blood pressure According to RUSHMER (1961) changes of the stroke volume and changes in the peripheral vascular resistance may be reflected in changes of arterial pulse pressure and diastolic arterial blood pressure, respectively Both heart rate and arterial pulse pressure decreased markedly after propranolol The arterial diastolic blood pressure did not change significantly The decrease of the heart rate was maximal already at the termination of the infusion of propranolol, while the decrease of the pulse pressure appeared to reach its maximum about 10 minutes after the termination The heart rate and pulse pressure were also reduced after H 56/28, but the decrease of these variables appeared to be less pronounced after H 56/28 than after propranolol The diastolic blood pressure was not significantly changed after H 56/28

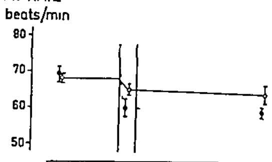
Discussion

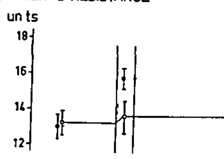
The results confirm studies in animals and man (ÅBLAD, BROGÅRD & EK 1967, JOHNSSON, NORRBY & SÖLVELL 1967) indicating that propranolol and H 56/28 are equipotent β adrenergic receptor antagonists after intravenous administration It has been shown that the β-receptor blocking action of H 56/28 is characterized by a time-effect curve practically identical to that of propranolol (JOHNSSON, NORRBY & SÖLVELL 1967, ÅBLAD *et al* 1967)

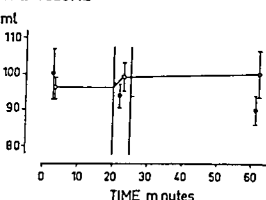
The recorded decrease of cardiac output after propranolol is in the same range as in previously reported studies (e g EPSTEIN *et al* 1965, HARRIS *et al* 1966 CUMMING & CARR 1966, SOWTON & HAMER 1966) A significant reduction of cardiac output was found already three minutes after the start of propranolol infusion This indicates that the action of the drug is characterized by a rapid onset which has previously been reported by EPSTEIN *et al* 1965 The reduced heart rate indicates a negative chronotropic action The reduced arterial pulse pressure and the tendency for a reduction of stroke volume and increase of mean right atrial pressure observed after administration of propranolol may suggest a negative inotropic action (cf ÅBLAD, BROGÅRD & EK 1967) These

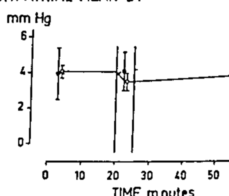












• •PROPRANOLOL ○—○H 56/28

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Discussion

The results confirm studies in animals and man (ÅBLAD, BROGÅRD & EK 1967, JOHNSSON, NORRBY & SÖLVELL 1967) indicating that propranolol and H 56/28 are equipotent β-adrenergic receptor *antagonists* after intravenous administration It has been shown that the β receptor blocking action of H 56/28 is characterized by a time effect curve practically identical to that of propranolol (JOHNSSON, NORRBY & SÖLVELL 1967, ÅBLAD *et al* 1967)

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1966, CUMM[illegible]
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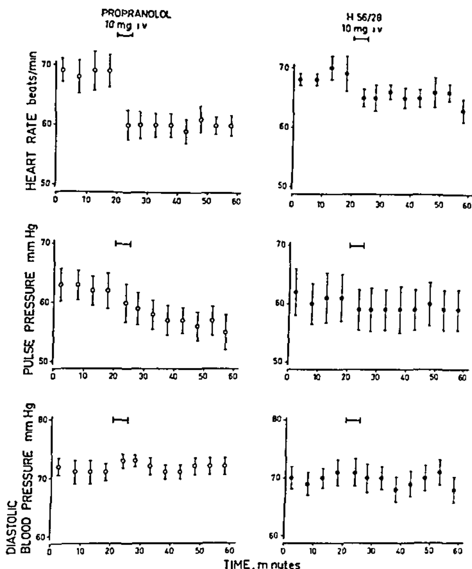


Fig 2 Effects of propranolol and H 56/28 (10 mg i v of the racemic forms) on heart rate, arterial pulse pressure and diastolic blood pressure Mean±s e m ($n=5$)

responses were probably due to a reduction of endogenous sympathetic tone through β-receptor blockade in the heart (cf ÅBLAD, BROGÅRD & EK 1967)

The decrease of cardiac output after propranolol was accompanied by an increase of total peripheral vascular resistance since mean arterial blood pressure was not significantly reduced It is likely that the greatest part of the increased peripheral vascular resistance observed after propranolol was due to an increase of vasoconstrictor nerve activity initiated by homeostatic pressure reflexes To some extent this peripheral vasoconstriction however might have been due to a reduction of an endogenous tone on vascular β receptors This is indicated by the regional vascular effects of intra arterially administered propranolol in man (JOHNSSON 1967)

From the unchanged cardiac output after administration of H 56/28 one might suspect that the agent does not affect the positive chronotropic and inotropic action of cardiac sympathetic nerve activity However, animal studies show unequivocally that H 56/28 and propranolol are equipotent antagonists of β-receptors in heart, whether these are activated by isoprenaline injections or by electrical stimulation of cardiac sympathetic nerves (ÅBLAD, BROGÅRD & EK 1967) A probable explanation of the difference in the effects of propranolol and H 56/28 observed in the present study is suggested by other data in the previously mentioned animal study (ÅBLAD, BROGÅRD & EK 1967) These investigators found that H 56/28 produced a positive chronotropic and inotropic effect in reserpinized, vagotomized, adrenalectomized and anaesthetized cats This effect could be inhibited by propranolol in low doses which, when given alone, did not affect cardiac function in this preparation These results indicate that H 56/28 possesses direct β-receptor stimulating properties and consequently could be defined as a partial agonist of β-receptors (STEPHENSON 1956), while propranolol has no β-receptor stimulating effects (BLACK DUNCAN & SHANKS 1965)

ÅBLAD BROGÅRD & EK (1967) further found that H 56/28 in intravenous doses between 0 05 mg/kg and 2 mg/kg, did not significantly affect cardiac rate and contractile force in vagotomized, anaesthetized cats without reserpine pretreatment In the same preparation propranolol elicited negative chronotropic and inotropic effects already after an intravenous dose of 0 05 mg/kg body weight These results indicate that H 56/28 in this animal preparation elicited a direct β-receptor stimulation of such a magnitude that the inhibitory effect of the drug on endogenous cardiac sympathetic tone was compensated over a wide dose range In view of these data it seems likely that in the present study the β adrenergic blocking effects of H 56/28 on the basal hemodynamics were antagonized by its β-receptor stimulating effects

In studies on reserpinized dogs, H 56/28 was found to exert a weak β-receptor stimulating action also on peripheral blood vessels, while propranolol was devoid of such a property (EK & JOHNSSON 1967—to be published) When given intra arterially to the human forearm, propranolol in a dose of 0 05 mg produced a small but significant decrease of the blood flow in the corresponding forearm (JOHNSSON 1967) The same intra arterial dose of H 56/28, however did not change forearm blood flow, probably because its β-receptor stimulating action compensated for its blockade of the endogenous tone on β-receptors in the forearm

It would thus appear that H 56/28 and propranolol have different effects not only on the heart but also on peripheral blood vessels Both these differences between the two β-adrenergic blocking agents may be explained by the fact that H 56/28 possesses an intrinsic β-receptor stimulating effect, while propranolol is devoid of such an effect

When H 56/28 or propranolol in the above-mentioned study (JOHNSSON 1967) was administered into a brachial artery in a high dose (0 5 mg) both drugs induced a vasodilatation in the corresponding forearm This effect, which was not due to a stimulation of the β receptors in the forearm, only appeared during and immediately after the administration of the drugs When H 56/28 or propranolol in the present study was administered intravenously in a relatively high dose (10 mg) no decrease of the total peripheral resistance or the arterial blood pressure occurred during the infusion Therefore it seems probable that the "direct" effect (not mediated via the β-receptors) of the β-blocking drugs on the peripheral blood vessels is of no importance in the dose used

Summary

The cardiovascular effects of a new β-adrenergic receptor blocking agent, H 56/28, have been compared to those of propranolol in 5 healthy persons The two agents (dose 10 mg i v) were equipotent blockers of the cardiovascular effects of isoprenaline, but they influenced the *basal hemodynamics* differently

Cardiac output decreased after propranolol (22±4 2 per cent) but not after H 56/28 Mean arterial blood pressure was not significantly altered by either agent

On the basis of results from animal studies with the two compounds it could be concluded that 1) propranolol reduced cardiac output by inhibiting endogenous sympathetic tone on the cardiac β receptors, 2) H 56/28 inhibited endogenous sympathetic tone on the heart to the same degree as propranolol but the hemodynamic consequences of this action of H 56/28 were overcome by a cardiac stimulation due to the slight "intrinsic" β-receptor stimulating action that this agent possesses

Acknowledgements

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Potency and Time-Effect Relationship in Man of Propranolol[1] and H 56/28[2]—Comparative Studies after Oral Administration

By

B ÅBLAD, G JOHNSSON, A NORRBY and L SOLVELL

In the development of a new drug it is of importance to determine the effect and potency in man as soon as possible Moreover, it is of great interest to know the rate of onset and the duration of the effect of the new drug However, with many drugs these properties are difficult to evaluate on the basis of human pharmacology studies and also on the findings in the earlier phases of clinical investigations

For some types of drugs these aspects of drug action can be evaluated in man by relatively simple means This is valid for the β adrenergic receptor antagonists where the activity of the drugs can be determined by testing their influence on the cardiovascular response to isoprenaline

In a previous investigation we were able to demonstrate the β receptor blocking properties of propranolol and H 56/28 by their influence *on the* effects of repeated intravenous infusions of isoprenaline on heart rate, pulse pressure and diastolic pressure (JOHNSSON *et al* 1966) In the present study the same technique was used to quantitate the β-receptor blocking properties of orally administered racemic H 56/28 and propranolol. Moreover the β receptor blocking effect of the optical isomers of H 56/28 has been studied

Methods

All studies were made as cross-over studies in normal subjects 20–25 years of age (weight range 72–80 kg) In each series all subjects received 2 or 3 β-receptor blocking agents on separate days The interval between each study was at least 3 days The studies started in the morning and all subjects had fasted over *night* They were in supine position *during*

[1] Inderal®
[2] Aptin®

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the whole study and a polyethylene catheter was inserted in an antecubital vein The systolic and diastolic arterial blood pressures were measured with the conventional auscultatory method The diastolic blood pressure was recorded when the sound became muffled The heart rate was recorded by ECG

The studies started after 30 minutes' rest with continuous measurements of arterial blood pressure and heart rate When a steady state level was reached isoprenaline was infused during 5 minutes in a dose of 0 02 μg l isoprenaline sulphate per kg body weight per minute (in one of the experimental series the corresponding dose of l isoprenaline sulphate was 0 03 μg) The isoprenaline was dissolved in isotonic saline containing 0 01 per cent ascorbic acid as a preservative The solution was given by a motor driven syringe at a constant rate (1 ml per minute)

The β adrenergic blocking drugs were given orally in randomized order as uncoated tablets of the same size and colour The disintegration time of the tablets was 6 minutes (according to B P 1963) The tablets were ingested with 100 ml of tap water 10 minutes after the end of the first infusion of isoprenaline The infusion was repeated 30 minutes after the intake of the tablets and thereafter every 35th minute Altogether 8 or in one series 7 infusions of isoprenaline were given

The *basal values* of blood pressure and heart rate in the results represent the mean values of ten measurements during the last 10 minutes before each infusion of isoprenaline The *effects of isoprenaline* on blood pressure and heart rate are represented by the mean values of three measurements during the last 2½ minutes of the infusion when the effect reached a steady state level

For statistical analysis of the results the *t* test (FISHER 1958) was employed The results are given as mean values and standard errors of the means (s e m)

Results

1 *Effects of racemic propranolol and racemic H 56/28 (20 mg)*

Animal investigations (ÅBLAD, BROGÅRD & EK 1967) and studies in man (FORSBERG & JOHNSSON 1967, JOHNSSON, NORRBY & SÖLVELL 1967) have shown that intravenously administered racemic propranolol and H 56/28 are equipotent β-blocking agents In the present study the effect of equal oral doses of the two drugs were compared The compounds were given as two 10 mg tablets Two studies were performed on a double blind basis in each of 4 subjects

The results are given in fig 1 Isoprenaline increased the heart rate and arterial pulse pressure while the diastolic blood pressure decreased These effects of isoprenaline were reduced after the administration of racemic propranolol and H 56/28 Propranolol, however, blocked the isoprenaline response more than H 56/28 in all three parameters For both drugs the effect was evident within 30 minutes after the administration, maximal after 1 to 3 hours and persisted for more than 4 hours

After propranolol the basal heart rate and basal pulse pressure decreased in each of the four subjects The mean decreases during maximal β-receptor blockade 1 to 3 hours after the administration of this drug were 6±3 0 beats/min and 7±2 0 mm Hg ($p<0\ 05$) respectively After H 56/28 there was a

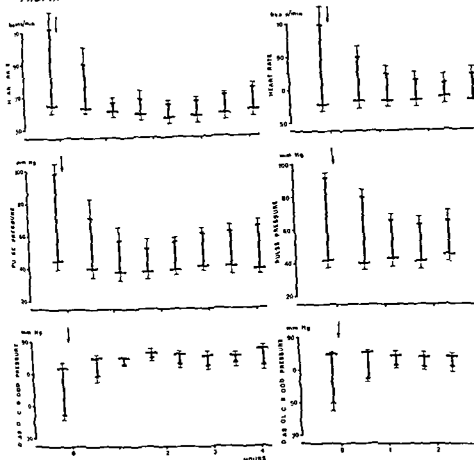


Fig 1 Effects of isoprenaline before and after racemic propranolol and H 56/28 (20 mg orally) on heart rate pulse pressure and diastolic blood pressure Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m. ($n=4$)

tendency to an increase of the basal heart rate (3±1 3 beats/min) and the pulse pressure (2±0 3 mm Hg) The basal diastolic blood pressure increased after propranolol (8±1 7 mm Hg $p<0$ 02), while it was essentially unchanged after H 56/28 (−1±2 9 mm Hg)

2 *Effects of racemic propranolol (10 and 20 mg) and H 56/28 (20 and 40 mg)*

Since orally administered propranolol in man was found to be more potent than H 56/28 as a β-adrenergic antagonist, studies were made to find the

Table 1

Drug	Oral dose mg	Per cent blockade of isoprenaline response		Effect on "basal heart rate (beats/min)	Effect on "basal" pulse pressure (mm Hg)
		Heart rate	Pulse pressure		
Propranolol	10	64	51	−6	−7
Propranolol	20	83	75	−7	−4
H 56/28 racemate	20	67	54	+1	−1
H 56/28 racemate	40	86	74	−1	−5

Effects of racemic propranolol and H 56/28 on basal heart rate and pulse pressure and the per cent blockade of the isoprenaline responses The effect of the drugs on the basal values are calculated as the difference between the basal values before the drugs were administered and the mean basal values recorded 1 to 3 hours after the administration of the drugs The per cent blockade of the effects of isoprenaline are calculated by relating the mean isoprenaline responses recorded 1 to 3 hours after the administration of propranolol or H 56/28 to the control responses registered before administration of the antagonists (mean of two experiments)

equipotent doses of the two drugs The studies were made in 2 subjects, each receiving 10 and 20 mg of propranolol and 20 and 40 mg of H 56/28 orally The results (table 1) indicate that the two drugs produced equal blockade of the isoprenaline response when the dose of H 56/28 was twice that of propranolol

Also in this study the same difference in the effect of the drugs on the basal heart rate and the basal pulse pressure was observed

3 *Effects of the levo and dextro forms of H 56/28 (20 mg)*

Animal studies have shown that the β-adrenergic blocking activity of H 56/28 mainly resides in the levo isomer, while the dextro isomer only has a weak β-adrenergic blocking effect (ÅBLAD, BROGÅRD & EK 1967)

The aim of the present investigation was to study the β-receptor blocking potency of the two isomers in man A double blind study was performed in 4 subjects, each of whom received the two substances as two 10 mg tablets

The results are given in fig 2 The response to isoprenaline was only slightly affected by the dextro form but was considerably reduced by the levo form The time-effect relationship for the levo form of H 56/28 was the same as for the racemic H 56/28, i e the β-adrenergic blocking effect was evident within 30 minutes, maximal after 1 to 3 hours and persisted for more than 4 hours after the administration The per cent blockade by 20 mg of the levo form of H 56/28 was found to be almost the same as that obtained with 20 mg of racemic propranolol (see table 1) Thus 20 mg of the levo form of

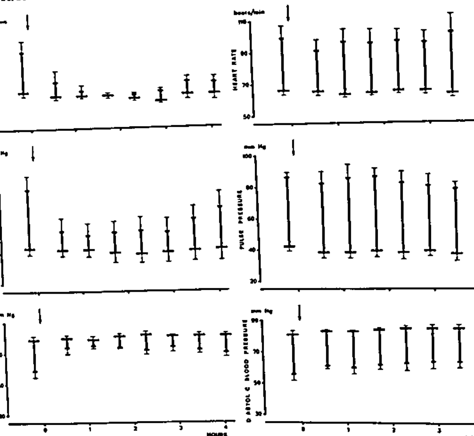


Fig 2. Effects of isoprenaline before and after the levo and dextro forms of H 56/28 (20 mg orally) on heart rate, pulse pressure and diastolic blood pressure Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m. ($n=4$)

H 56/28 produced 88 per cent blockade of the heart rate and 66 per cent blockade of the pulse pressure responses (calculated from the values obtained 1–3 hours after the administration of the tablets) There were no significant effects of the levo or the dextro isomers of H 56/28 on the basal heart rate, pulse pressure or diastolic blood pressure

4 *Effects of racemic propranolol (10 mg), racemic H 56/28 (20 mg) and the levo form of H 56/28 (10 mg)*

To confirm the findings in the above mentioned separate studies of probable equipotent dosages of racemic propranolol, racemic H 56/28 and the levo

Table 1

Drug	Oral dose mg	Per cent blockade of isoprenaline response		Effect on "basal" heart rate (beats/min)	Effect on "basal" pulse pressure (mm Hg)
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The aim of the present investigation was *to study the β-receptor blocking* potency of the two isomers in man A double blind study was performed in 4 subjects, each of whom received the two substances as two 10 mg tablets

The results are given in fig 2 The response to isoprenaline was *only slightly* affected by the dextro form but was considerably reduced by the levo form The time-effect relationship for the levo form of H 56/28 was the same as for the racemic H 56/28, i e the β-adrenergic blocking effect was evident within 30 minutes, maximal after 1 to 3 hours and persisted for more than 4 hours after the administration The per cent blockade *by 20 mg of the levo* form of H 56/28 was found to be almost the same as that obtained with 20 mg of racemic propranolol *(see table 1)* Thus 20 mg of the levo form of

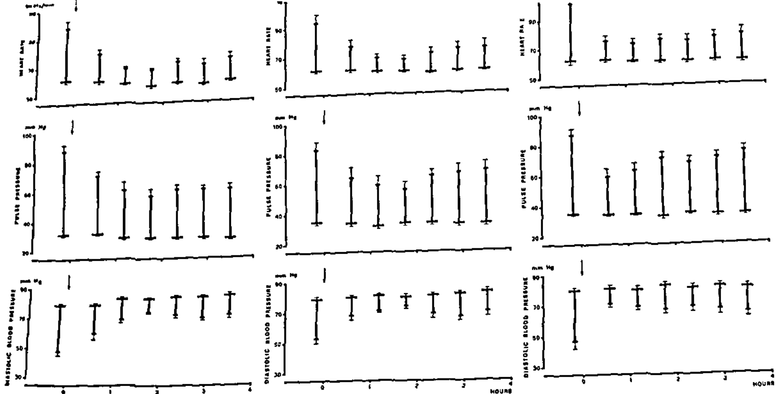


Fig 3 Effects of isoprenaline *before* and *after* racemic propranolol (10 mg orally), racemic H 56/28 (20 mg orally) and the levo form of H 56/28 (10 mg orally) on heart rate, pulse pressure and *diastolic* blood pressure Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m ($n=8$).

form of H 56/28, the following more extensive study was designed The same oral doses of racemic propranolol and the levo form of H 56/28 (10 mg) were compared to double the dose of racemic H 56/28 (20 mg) Four subjects received each drug two times on a double blind basis in a randomized order Six studies were thus performed in each subject The experimental design was the same as in the preceeding studies with two exceptions isoprenaline was infused during seven periods instead of eight and the dose of isoprenaline was increased to 0 03 μg per kg body weight per minute

The results are shown in fig 3 and table 2 All three agents markedly reduced the effects of isoprenaline on heart rate and blood pressures As in the previous studies the β receptor blocking effect was evident within 30 minutes after the administration of the drugs and the blockade still persisted when the study was terminated 3½ hours after the administration No statistically significant differences were found between the 3 agents in the per cent blockade of heart rate, arterial pulse pressure or diastolic blood pressure The results in this study are thus in accordance with the previous preliminary results indicating that racemic H 56/28 has to be given in about twice the oral dose of the levo form and racemic propranolol to produce the same β-adrenergic receptor blockade After racemic propranolol the basal pulse pressure and heart rate decreased while the diastolic blood pressure increased but no significant changes were seen after racemic H 56/28 or the levo form of H 56/28

Table 2

Drug	Oral dose mg	Per cent blockade of isoprenaline response			Effect on "basal" heart rate (beats/min)	Effect on "basal" pulse pressure (mm Hg)	Effect on "basal" diastolic pressure (mm Hg)
		Heart rate	*Pulse* pressure	*Diastolic* pressure			
Propranolol	10	64±5 0	45±6 2	55±9 1	−4±1 0 $p<0 01$	−3±0 8 $p<0 01$	+5±1 6 $p<0 02$
H 56/28 racemate	20	59±8 4	39±3 9	57±4 9	−1±0 8	−2±1 1	+2±0 9
H 56/28 levo form	10	63±3 1	33±3 4	56±1 8	0±1 8	−1±1 2	+2±1 1

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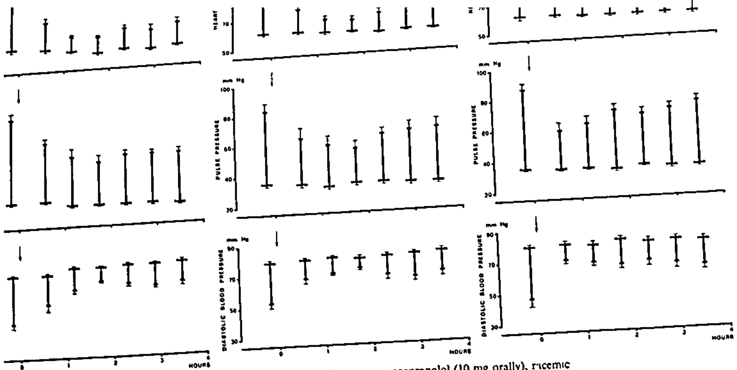


Fig 3 Effects of isoprenaline before and after racemic propranolol (10 mg orally), racemic H 56/28 (20 mg orally) and the levo form of H 56/28 (10 mg orally) on heart rate, pulse pressure and diastolic blood pressure Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m ($n = 8$).

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		Heart rate	Pulse pressure	Diastolic pressure			
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have not yet been clarified but might be explained by differences in e g absorption or different liver uptake

The basal heart rate and pulse pressure were consistently reduced after racemic propranolol but unchanged after equipotent β-receptor blocking doses of the racemic and the levo form H 56/28 These observations may suggest that propranolol evoked small negative chronotropic and inotropic effects on the heart while H 56/28 was devoid of such effects This interpretation is supported by the findings of FORSBERG & JOHNSSON (1967) These authors made a comparative study of the effects of racemic propranolol and H 56/28 on basal hemodynamics in healthy subjects by more direct methods They found that propranolol (10 mg i v) reduced basal cardiac output by 22 per cent on an average while no significant change could be observed after H 56/28 (10 mg i v)

Animal studies indicate that this difference can be explained by the fact that H 56/28 (i e its levo isomer) has a small β receptor stimulating activity while propranolol is devoid of such a property (ÅBLAD, BROGÅRD & EK 1967)

In view of the animal data it is reasonable to presume that in the present study the reduced basal heart rate and pulse pressure after racemic propranolol were due to inhibition of the endogenous sympathetic tone on the heart Racemic H 56/28 and its levo form on the other hand did not change the basal heart rate and pulse pressure probably because it elicited a direct cardiac β receptor stimulation that compensated for the inhibitory effect of the drug on the endogenous cardiac sympathetic tone

The basal arterial diastolic blood pressure increased after racemic propranolol but was essentially unchanged after racemic H 56/28 and its levo isomer This increase of the arterial diastolic blood pressure after propranolol probably reflects an increase of the peripheral vascular resistance Forsberg and Johnsson in the above mentioned study recorded an increase of the vascular peripheral resistance after propranolol but not after H 56/28 The authors suggested that this effect of propranolol was largely due to a reflex increase of vasoconstrictor nerve activity secondary to the reduction of cardiac output

Propranolol is currently the only β receptor blocking drug in the market A definite drawback with this drug is its tendency to elicit cardiac failure (STEPHEN 1966) probably due to elimination of the basal endogenou[illegible] [illegible] this question is now being investigated

Summary

The potency and time-effect relationship of an orally administered new β-adrenergic receptor antagonist, H 56/28, was studied in man by recording heart

Discussion

Isoprenaline primarily activates the adrenergic β-receptors and elicits positive cardiac chronotropic and inotropic responses and peripheral vasodilatation (AHLQUIST 1965) The positive cardiac inotropic effect and the reduction of peripheral vascular resistance may be reflected in an increase of arterial pulse pressure and a decrease of diastolic arterial blood pressure respectively (RUSHMER 1961) The circulatory effects of an isoprenaline infusion are rapidly induced and also subside rapidly after cessation of the infusion Furthermore isoprenaline induces the same circulatory responses during repeated infusions (JOHNSSON *et al* 1966) Therefore repeated administrations of isoprenaline can be used for determinations of the potency and the time-effect relationship of β-adrenergic antagonists in man

In the present investigation racemic H 56/28, its optical isomers and racemic propranolol were compared with this technique

The results in man confirm the findings in animal studies that racemic H 56/28, its levo form and racemic propranolol given orally are effective β-adrenergic antagonists The dextro form of H 56/28 showed only weak β-receptor blocking activity in the dose given (20 mg) This is in accordance with the findings in animal studies that the dextro form of H 56/28 is about 100 times weaker than the levo form as a β-receptor blocking agent (ÅBLAD, BROGÅRD & EK 1967) Racemic propranolol has recently been resolved into its optical isomers and the levo form has also been demonstrated to have the most potent β-receptor blocking activity (HOWE & SHANKS 1966)

The time-effect relationships of H 56/28 (racemate and levo form) and racemic propranolol were found to be similar A marked β-receptor blocking activity could be observed in all subjects as early as 30 minutes after the oral administration The most marked blocking activity was seen between 1 to 3 hours after the ingestion of the drugs Thereafter the effect decreased but there was still a pronounced β-receptor blocking activity at the termination of the studies about 4 hours after the oral intake No side effects were noted in any of the subjects after the administration of the β-receptor blocking drugs

Quantitatively the β-receptor blocking potency of orally administered racemic H 56/28 was equal to half the dose of racemic propranolol The levo form of H 56/28 had about the same potency as racemic propranolol However, animal and human studies with intravenous administrations have shown equal potency of racemic H 56/28 and propranolol (ÅBLAD, BROGÅRD & EK 1967, FORSBERG & JOHNSSON 1967, JOHNSSON, NORRBY & SÖLVELL 1967), while half the dose of the levo form of H 56/28 induced the same β receptor blocking activity The reasons for the discrepancy in potency relationship between propranolol and H 56/28 after oral and intravenous administrations

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From the Departments of Pharmacology and Internal Medicine II,
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Potency and Time-Effect Relationship in Man of Propranolol[1] and H 56/28[2]

I. Comparative Studies after Intravenous Administration
II. Studies on the Ratio of Equipotent Oral and Intravenous Doses

By

G JOHNSSON, A. NORRBY and L SÖLVELL

Both the racemic and levo forms of orally administered H 56/28 have been shown to be potent β adrenergic receptor blocking drugs in man Comparative studies with racemic propranolol, racemic H 56/28 and the levo form of H 56/28 have shown that the levo form of H 56/28 was equipotent to racemic propranolol while racemic H 56/28 had to be given in the double dose to give an equivalent β receptor blockade (ÅBLAD *et al* 1967) The drugs were administered orally on a double blind basis

The aim of the present investigation was to study the potency and time-effect relationship in man of intravenously administered racemic propranolol, H 56/28 and the levo form of H 56/28 Moreover, the β-receptor blocking effects of orally and intravenously administered racemic H 56/28 and the levo form of H 56/28 were compared in order to determine equipotent oral and intravenous doses in man

The β-receptor blocking effect was evaluated in normal subjects by giving repeated intravenous infusions of isoprenaline according to the method of ÅBLAD *et al* (1967) Isoprenaline was administered before and repeatedly after the administration of the β-receptor blocking drugs, the effects of isoprenaline on heart rate, pulse pressure and diastolic blood pressure were measured

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rate and blood pressure responses to repeated intravenous isoprenaline infusions Comparative studies were made with propranolol on a double blind basis Moreover the optical isomers of H 56/28 were studied with the same technique

Racemic H 56/28, its levo form and racemic propranolol were found to be effective β-adrenergic antagonists (dose range 10–40 mg) The approximate relationship of equipotent oral doses was found to be 2 1 1 respectively All three agents showed the same time-effect relationship and the effect was evident within 30 minutes, maximal after 1 to 3 hours and persisted for more than 4 hours after the administration The dextro form of H 56/28 showed only a weak β-receptor blocking activity in the dose given (20 mg)

The basal heart rate and pulse pressure were reduced after racemic propranolol but unchanged after equipotent β-receptor blocking doses of H 56/28 (racemic and levo form) The cause of this difference is discussed and it is suggested to be related to a slight sympathomimetic action on the heart of H 56/28 while propranolol is devoid of such a property

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[2] Aptin®

Methods

All studies were conducted in healthy male subjects 20–25 years of age by the double blind method The weight of the subjects ranged from 64 to 80 kg Each subject in each series received the β-receptor blocking drugs in a randomized order on separate days The interval between each study on the same subject was at least 3 days The β receptor blocking drugs were given as intravenous solutions or as tablets of the same size and colour The disintegration time of the tablets was 6 minutes (according to B P 1963) The studies were made in the morning after the subjects had fasted over night The subjects were in a supine position during the whole study For the intravenous administration of the drugs a polyethylene catheter was inserted in an antecubital vein The systolic and diastolic blood pressures were measured with the conventional auscultatory method The diastolic pressure was measured when the sound became muffled The heart rate was recorded by ECG

After about 20 minutes' rest the studies commenced with measurements of arterial blood pressure and heart rate Ten minutes later isoprenaline was infused by a motor driven syringe during 5 minutes in a dose of 0 03 μg l-isoprenaline sulphate per kg body weight per minute Isoprenaline was dissolved in isotonic saline containing 0 01 per cent ascorbic acid and the infused volume was 0 5 ml per minute

In the *intravenous* studies the β-receptor blocking drugs were infused between the 30th and the 35th minute after the first infusion of isoprenaline The total dose of intravenously administered racemic propranolol and H 56/28 was 5 mg and the dose of the levo form of H 56/28 was 2 5 mg The infused volume was one ml per minute Ten minutes after the termination of the infusion of the β-adrenergic blocking drugs an intravenous infusion of isoprenaline was given during 5 minutes at a rate of 1 5 ml per minute corresponding to a dose of 0 09 μg l-isoprenaline sulphate per kg body weight per minute Thus, this dose of isoprenaline was 3 times higher than the first dose

The higher dose of isoprenaline was chosen since preliminary studies had indicated that racemic H 56/28 and propranolol in the dose range used in these series almost completely abolished the circulatory effects of 0 03 μg per kg body weight per minute of l-isoprenaline sulphate (cf JOHNSSON *et al* 1966)

Infusions of l-isoprenaline sulphate (0 09 μg per kg body weight per minute) were then repeated every 35th minute Altogether 5 infusions of isoprenaline were performed

In the studies with the *oral* administration of the β adrenergic blocking drugs the tablets were given with 100 ml tap water 10 minutes after the termination of the initial infusion of isoprenaline (0 03 μg per kg body weight per minute) The dose of racemic H 56/28 was 50 mg and that of the levo

form was 25 mg The first infusion of the higher dose of isoprenaline (0 09 μg per kg body weight per minute) was performed 45 minutes after the administration of the β-blocking drugs It was then repeated 3 times with 35 minutes intervals

In a preliminary oral study (series 2 in the Results) with racemic H 56/28 (100 mg) and the levo form of H 56/28 (50 mg) the higher dose of isoprenaline was infused three times (every 60th minute) after the administration of the β adrenergic receptor blocking drugs

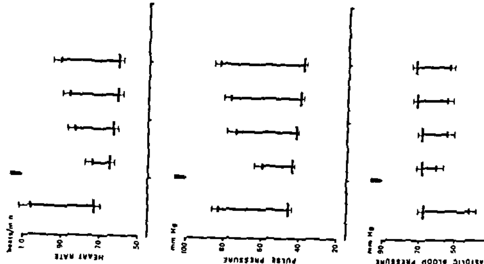
The *basal values* of heart rate, pulse pressure and diastolic blood pressure in the tables and figures represent the mean value of 10 measurements on each subject made during the last 10 minutes before each infusion of isoprenaline The *effects of isoprenaline* on heart rate, pulse pressure and diastolic blood pressure are represented by the mean values of three measurements during the last 2½ minutes of the isoprenaline infusion when the effect had reached a steady state level The results are given as mean values and standard errors of the means (s e m) For statistical analysis of the results the *t* test was employed (FISHER 1958)

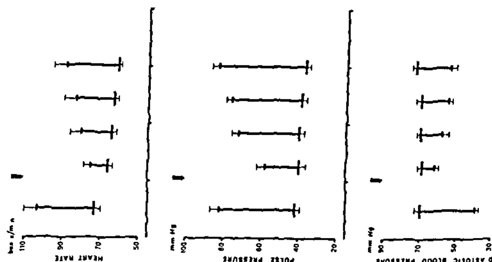
Results

1 *Effects of intravenously administered racemic H 56/28 (5 mg), the levo form of H 56/28 (2 5 mg) and racemic propranolol (5 mg)*

Animal studies have shown that intravenously administered racemic H 56/28 and racemic propranolol were equipotent β-receptor blocking substances (ÅBLAD BROGÅRD & EK, 1967) Studies of the optical isomers of H 56/28 have demonstrated that the main β-receptor blocking activity resided in the levo form and that the dextro form had only weak receptor blocking properties (ÅBLAD, BROGÅRD & EK 1967, ÅBLAD *et al* 1967) Therefore in the present study racemic H 56/28 and propranolol were given in the same dose (5 mg) while the levo form of H 56/28 was given in half that dose (2 5 mg)

The studies were made in 5 subjects who each received the 3 drugs on a double blind basis The results are given *in fig 1* Although isoprenaline was administered in a 3 times higher dose after the β-adrenergic drugs the effects of isoprenaline on heart rate, pulse pressure and diastolic blood pressure were markedly reduced after all three β-receptor antagonists The most marked β-receptor blocking effect was recorded 10-15 minutes after the administration of the antagonists and decreased continuously thereafter The effect of adrenergic blockade on heart rate and diastolic blood pressure was still marked at the end of the study two hours after the administration of the β-receptor blocking drugs At the end of the study the same increase of the pulse pressure was found as before the β-blocking drugs However, it





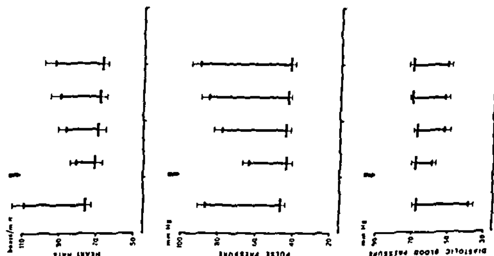


Fig 1. Effects of isoprenaline before and after intravenous administration of racemic H 56 28, its levo form and racemic propranolol on heart rate, pulse pressure and diastolic blood pressure The dose of isoprenaline was 3 times as great after the administration of the β blocking drugs as before Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m ($n=5$)

Table I Effects of intravenously administered racemic H 56/28, its levo isomer and racemic propranolol on basal heart rate pulse pressure and diastolic blood pressure The changes are calculated as the difference between the mean of the basal values during the 10-minute period immediately before the β-blocking drugs and the mean of the values during the basal period before and that after the maximal β-blocking effect. Mean±s e m. (n 5).

Drug	Intravenous dose mg	Decrease of heart rate beats/min	Decrease of pulse pressure mm Hg	Decrease of diastolic blood pressure mm Hg
H 56/28 racemate	5	2±1 5	2±1.5	1±0 9
H 56/28 levo form	2 5	3±1 5	1±1 2	1±0 6
Propranolol	5	5±1 6 $p<0\ 05$	4±1 2 $p<0\ 05$	0±1 4

is obvious that some blockade persisted at the end of the study, since isoprenaline was infused in a dose three times as great as before the administration of the β receptor blocking agents

No significant differences could be found between the three agents in the blockade of the effects of isoprenaline on heart rate, pulse pressure or diastolic blood pressure

After all three drugs the *basal* heart rate and pulse pressure tended to decrease continuously during the study while the diastolic blood pressure tended to increase These changes could hardly be explained only by an effect of the β receptor blocking properties of the drugs since the isoprenaline blockade was maximal already within 10–15 minutes after the administration of the drugs A contributary factor is probably the prolonged rest of the subjects with more basal conditions at the end of the study The effects of the β blocking drugs on the basal heart rate, pulse pressure and diastolic blood pressure are given in table I After racemic propranolol a significant decrease of the heart rate (5±1 6 beats per minute, $p<0\ 05$) and the pulse *pressure* (4±1 2 *mm Hg*, $p<0\ 05$) was found while no significant changes occurred after racemic H 56/28 and its levo form No significant changes of the diastolic blood pressure could be observed after the three drugs

2 *Effects of orally administered racemic H 56/28 (100 mg) and the levo form of H 56/28 (50 mg)*

In order to find equipotent oral and intravenous β-receptor blocking doses of the racemic and levo forms of H 56/28, the following preliminary study was performed in 2 subjects who each received 100 mg of racemic H 56/28 and 50 mg of the levo form of H 56/28 orally

Studies in man with different doses of orally administered H 56/28 have shown that the blockade of the isoprenaline response was maximal 1–3 hours after the administration of the β-receptor blocking drugs (ÅBLAD *et al* 1967) Thus, in the present series, the isoprenaline response was studied during this time

The results are given in fig 2 Both drugs markedly inhibited the effects of isoprenaline on heart rate, pulse pressure and diastolic blood pressure The β-receptor blocking effect was maximal one hour after the administration of both drugs and thereafter decreased during the study

When comparing the results in the present oral study with the results in the preceeding intravenous study (fig 2 and 1 respectively) it is evident that 100 mg of orally administered racemic H 56/28 and 50 mg of the levo form of H 56/28 reduced the circulatory effects of isoprenaline more than intravenously administered racemic H 56/28 and the levo form of H 56/28 (5 and 2 5 mg respectively)

3 *Effects of orally administered racemic H 56/28 (50 mg) and the levo form of H 56/28 (25 mg)*

The preceeding studies have shown that, when evaluated in terms of maximally observed blockade of isoprenaline response, the ratio of equipotent oral and intravenous doses was less than 20/l in the dose levels studied Therefore in this series racemic H 56/28 and its levo form were given orally in a dose 10 times higher than in the intravenous series

Each of 5 subjects received 50 mg of racemic H 56/28 and 25 mg of the levo form of H 56/28 on a double blind basis

The results are given in fig 3 The effects of isoprenaline on heart rate, pulse pressure and diastolic blood pressure were markedly reduced after both racemic H 56/28 and its levo form in spite of the three-fold dose of isoprenaline The β-blocking effect of the two drugs appeared to be most marked 1 to 1½ hours after the administration of both drugs The blockade effect on heart rate and diastolic blood pressure was still marked at the end of the study (2½ hours after the administration of the β-receptor blocking drugs) At this time the same increase of the pulse pressure was found as before the β-blocking drugs However, it is obvious that some blockade persisted at the end of the study as isoprenaline then was infused in a three-fold dose

No significant differences could be found between the two drugs in the blockade of the circulatory effects of isoprenaline

4 *Comparison of the potency of orally and intravenously administered H 56/28*

A comparison of the results in series 1 and 3 is given in table II The results are given as the responses of isoprenaline on heart rate, pulse pressure and diastolic blood pressure before the administration of the β receptor blocking

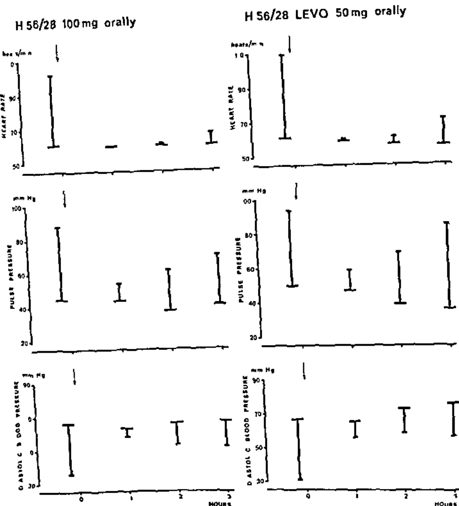


Fig 2 Effects of isoprenaline before and after oral administration of racemic H 56/28 (100 mg) and its levo form (50 mg) on heart rate, pulse pressure and diastolic blood pressure The dose of isoprenaline was 3 times as great after the administration of the β blocking drugs as before Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values of two experiments

drugs and responses to isoprenaline during maximal blockade It is evident that 50 mg of orally administered racemic H 56/28 reduced the circulatory effects of isoprenaline to about the same extent as 5 mg administered intravenously About the same reduction of the isoprenaline responses was seen after the corresponding oral and intravenous doses of the levo form of H 56/28 (25 and 2 5 mg respectively) No significant differences were found between the racemic and the levo form of H 56/28 in the doses given

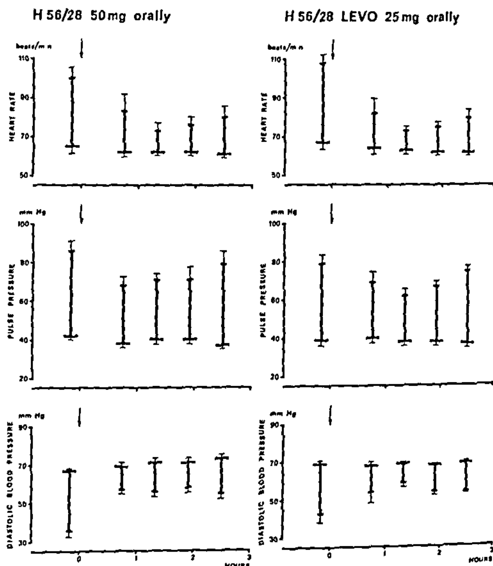


Fig 3 Effects of isoprenaline before and after oral administration of racemic H 56 28 (50 mg) and its levo form (25 mg) on heart rate, pulse pressure and diastolic blood pressure The dose of isoprenaline was 3 times as great after the administration of the β blocking drugs as before Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m ($n=5$)

There were no indications that, when evaluated in terms of maximally observed blockade of isoprenaline response, the ratio between oral and intravenous doses was less than 10/1 as the blockade tended to be higher after the intravenous than after the oral doses In series 2 it was shown that the ratio between oral and intravenous doses was definitely less than 20/1 Thus the ratio of equipotent oral and intravenous doses for the racemic and levo forms of H 56/28 seems to be about 10/1 or slightly higher for both substances in the dose levels studied

able II Initial response to isoprenaline and the response to isoprenaline during the infusion period with the maximal blockade after orally and intravenously administered racemic H 56/28 and its levo form Mean±s e m (n 5)

Drug	Route of administration	Dose mg	Heart rate (beats/min)		Pulse pressure (mm Hg)		Diastolic blood pressure (mm Hg)	
			Initial response to isoprenaline	Response to isoprenaline during maximal blockade	Initial response to isoprenaline	Response to isoprenaline during maximal blockade	Initial response to isoprenaline	Response to isoprenaline during maximal blockade
H 56/28 racemate	i v	5	33±5 7	10±0 9	40±2 5	20±3 0	29±2 1	9±2 8
H 56/28 racemate	oral	50	35±4 0	11±2 6	44±4 1	30±5 3	31±2 6	12±1 8
H 56/28 levo form	i v	2 5	30±4 2	9±1 4	40±3 4	18±1 4	31±2 0	7±1 8
H 56 28 levo form	oral	25	41±4 5	10±1 6	40±2 0	24±2 9	26±3 5	10±1 7

Discussion

Isoprenaline primarily activates the adrenergic β-receptors and elicits positive chronotropic responses positive inotropic responses and peripheral vasodilation (AHLQUIST 1965) The positive inotropic effect and the reduction of peripheral vascular resistance may be reflected in an increase of arterial pulse pressure and a decrease of arterial diastolic pressure respectively (RUSHMER 1961) The circulatory effects of an isoprenaline infusion are rapidly induced and also subside rapidly after cessation of the infusion Moreover, repeated infusions give the same circulatory response (JOHNSSON et al 1966) Therefore, a technique with repeated infusions of isoprenaline is suitable for studies on potency and time-effect relationships of β-adrenergic antagonists

In previous studies the same dose (0 02 or 0 03 μg per kg body weight per minute) of isoprenaline was infused both before and after the β adrenergic blocking agents (JOHNSSON et al 1966, ÅBLAD et al 1967) In the present investigation relatively higher doses of the β-receptor blocking drugs were given These doses of the β-receptor blocking drugs will almost totally block the circulatory effects of isoprenaline if 0 02 or 0 03 μg per kg body weight per minute is used (cf JOHNSSON et al 1966) A total blockade of the isoprenaline response makes it more difficult to compare β adrenergic antagonists quantitatively Thus a higher dose of isoprenaline was given after the β-receptor blocking drugs (0 09 μg per kg body weight per minute) The same high dose of isoprenaline both before and after the administration of the β receptor

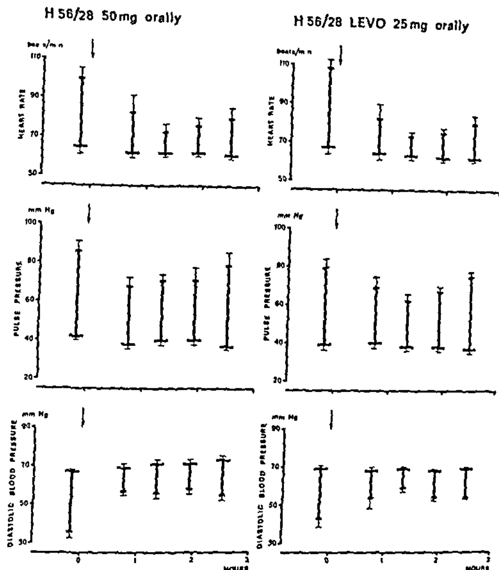


Fig 3 Effects of isoprenaline before and after oral administration of racemic H 56 28 (50 mg) and its levo form (25 mg) on heart rate pulse pressure and diastolic blood pressure The dose of isoprenaline was 3 times as great after the administration of the β blocking drugs as before Each bar represents the change from the basal value induced by isoprenaline The results are given as mean values and s e m ($n = 5$)

There were no indications that, when evaluated in terms of maximally observed blockade of isoprenaline response, the ratio between oral and intravenous doses was less than 10/l as the blockade tended to be higher after the intravenous than after the oral doses In series 2 it was shown that the ratio between oral and intravenous doses was definitely less than 20/l Thus the ratio of equipotent oral and intravenous doses for the racemic and levo forms of H 56/28 seems to be about 10/l or slightly higher for both substances in the dose levels studied

vious results obtained after oral and intravenous administrations (ÅBLAD *et al* 1967, FORSBERG & JOHNSSON 1967) The different influence of the drugs on the basal heart rate and pulse pressure may be ascribed to the fact that racemic H 56/28 and its levo form have slight β-receptor stimulating properties while propranolol lacks this effect. (cf ÅBLAD, BROGÅRD & EK 1967)

Summary

The potency and time-effect relationships of intravenously administered racemic H 56/28, the levo form of H 56/28 and racemic propranolol were studied in man by recording heart rate and blood pressure responses to repeated intravenous infusions of isoprenaline Moreover, the β-receptor blocking effect of orally and intravenously administered racemic H 56/28 and its levo form were compared in order to determine equipotent oral and intravenous doses in man

Racemic H 56/28 (5 mg), its levo form (2 5 mg) and racemic propranolol (5 mg) were found to be potent β adrenergic antagonists The ratios of equipotent intravenous doses were found to be about 2 1 2 All three agents had the same time-effect relationship and the effect was maximal within 10–15 minutes after the administration and still persisted at the end of the study, 2 hours later

Racemic H 56/28 had to be given orally in a dose of 50 mg to produce the same maximal β-receptor blockade as 5 mg intravenously The corresponding doses for the levo form of H 56/28 were 25 and 2 5 mg respectively Thus the ratio of equipotent oral and intravenous doses, when evaluated in terms of maximally observed blockade of isoprenaline response, was about 10/1 for both drugs in the dose levels used

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blocking drugs was not used, as the effects of isoprenaline before β-receptor blockade could be expected to be uncomfortable to the subjects

In the present investigation the β-receptor blocking effects in man of intravenously administered racemic H 56/28, its levo form and racemic propranolol were compared All substances were found to be potent β-adrenergic antagonists and the time-effect relationships of H 56/28 (racemate and levo form) and racemic propranolol were found to be the same The blocking effect was evidently characterized by rapid onset since the effects of the isoprenaline infusion given 10–15 minutes after the drug administration were antagonized most markedly The antagonistic activity then decreased succesively but there was still a pronounced blockade 2 hours after the administration

Quantitatively the β-receptor blocking potency of intravenously administered racemic H 56/28 and propranolol was about the same Half the dose of the levo form of H 56/28 had the same β-receptor blocking activity Thus the relationships of equipotent intravenous doses of racemic H 56/28, its levo form and racemic propranolol were 2 1 2 These results are in agreement with previous findings in animals (ÅBLAD, BROGÅRD & EK 1967) It has previously been shown also in man that the β-blocking activity of H 56/28 is mainly exerted by its levo isomer (JOHNSSON *et al* 1966, ÅBLAD *et al* 1967)

One main purpose of the present investigation was to find equipotent β-receptor blocking doses of orally and intravenously administered racemic H 56/28 and its levo form Studies in 2 subjects showed that 100 mg of orally administered racemic H 56/28 and 50 mg of its levo form reduced the circulatory effects of isoprenaline to a greater extent than 5 mg intravenously of the racemate and 2 5 mg of the levo form However, 50 mg of orally administered racemic H 56/28 and 25 mg of its levo form were, when evaluated in terms of maximally observed blockade of isoprenaline response, about equipotent to the above mentioned intravenous doses Thus the ratios of equipotent oral and intravenous doses were about 10/1 for both drugs in the dose levels used In a previous investigation it was found that racemic H 56/28 had to be given orally in a dose two times as great as racemic propranolol to produce the same β-receptor blockade (ÅBLAD *et al* 1967) In the present intravenous studies they were found to be about equipotent Therefore it may be deduced that the corresponding ratio of equipotent oral and intravenous doses for racemic propranolol should be about 5/1

The time for maximal β receptor blocking effect after oral and intravenous administrations of the β-receptor blocking drugs differed The β receptor blocking effect after intravenous administration was maximal within 10–15 minutes and after oral administration within 1–1½ hours

A significant decrease of the basal heart rate and the pulse pressure was observed after intravenously administered racemic propranolol but not after racemic H 56/28 or its levo form These findings are in accordance with pre-

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BORGENS FORLAG COPENHAGEN 1967

Denne afhandling er i forbindelse med omstående tidligere publicerede afhandlinger af det lægevidenskabelige fakultet ved Københavns Universitet antaget til offentligt at forsvares for den medicinske doktorgrad

København, den 30 januar 1967

MOGENS FABER
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Translated from the Danish
by Harry Cowan, B Sc

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Previous Publications

A Torkell Jóhannesson Morphine as an inhibitor of brain cholinesterases in morphine tolerant and non tolerant rats *Acta pharmacol et toxicol* 1962, 19, 23–35

B Torkell Jóhannesson The concentrations of morphine in the brains of rats receiving morphine and neostigmine *Acta pharmacol et toxicol* 1962, 19, 286–292

C Torkell Jóhannesson & Jens Schou Morphine and normorphine in the brains of rats given identically analgaesic doses of morphine, codeine or normorphine *Acta pharmacol et toxicol* 1963, 20, 165–173

D Torkell Jóhannesson & Jens Schou Analgesic activity and brain concentration of morphine in tolerant and non tolerant rats given morphine alone or with neostigmine *Acta pharmacol et toxicol* 1963, 20, 213–221

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I Torkell Jóhannesson Larry A Rogers, James R. Fouts & L. A Woods Tolerance to morphine and codeine analgesia and hepatic enzymic microsomal drug metabolism in the rat. *Acta pharmacol et toxicol* 1965, 22 255–269

J Torkell Jóhannesson & L. A Woods Interaction of the analgesic effects of morphine and codeine in rats *Nature* 1965, 205, 811–812

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TO MY PARENTS

Foreword

The author's experimental work with morphine and codeine, which is published in the 10 above listed papers and on which the present study is directly based, was begun in January 1960 in The Department of Pharmacology, The University of Copenhagen, and completed in November 1964 in The Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa However, other duties during these years resulted in the work being interrupted for longer or shorter periods of time The author owes a great debt of gratitude to his chiefs, Professor Knud O Møller of Copenhagen, and Professor Laureen A Woods of Iowa City He is also deeply grateful to his present chief, Professor Kristinn Stefansson, The University of Iceland, Reykjavik, and to Professor Jens S Schou, the present head of The Department of Pharmacology, Copenhagen, and a friend of long standing for their unfailing help and encouragement during these years The author also wishes in particular to thank Professor [illegible] Møller for the [illegible] in December [illegible] The Univer-[illegible]

During the years, the author has had the great fortune to be able to conduct research pertinent to morphine and morphine like substances in close cooperation with many well known scientists, and to whom [illegible] is due Dr [illegible] thers and P[illegible] A Woods, [illegible] Professor James A Fouts and Dr Larry A Rogers at Iowa City The author also wishes to thank The Icelandic Scientific Foundation, The Nordisk Insulin Fund, The Danish Fund for the Promotion of Medical Research The State Department of The United States (a Fulbright Fellowship) and The Alfred Benzon Memorial Fund in Iceland for several research grants In particular, the author wishes to thank Director Johannes Nielsen Copenhagen for personal stipends towards both the expenses of the present publication in part as well as living expenses during previous years

Dr Eigill Hvidberg and cand pharm [illegible]

Contents

the text of this study Above all, the author wishes to thank his friend, Professor Jens S Schou, for his thorough and constructive criticism of the Danish manuscript

Finally, the author would like to thank Dr Sigurður Sigurðsson, The *Director General of Health, Reykjavík, Dr Jens Hald and Dr Egill Snorrason*, both of Copenhagen, for many helpful discussions and encouragement during by-gone years, and Mr Jørgen-Jensen of The Borgen Publishing Company, Copenhagen, and his wife, Karin, for all their valuable help and friendship

London W 2, the 8th of June, 1967

Torkell Jóhannesson

The analgesic effect in tolerant and non-tolerant rats

by

Torkell Jóhannesson

INTRODUCTION

In the vegetable kingdom there are numerous basic, nitrogen-containing substances which traditionally have been named alkaloids In addition to morphine and codeine, opium contains almost 30 such substances (cf REYNOLDS & RANDALL 1957) *Morphine* was the first alkaloid to be definitely isolated and studied This pioneering work was done by the German pharmacist FRIEDRICH WILHELM ADAM SERTURNER (born 1783, died 1841), whose experiments are discussed in greater detail below *Codeine* was isolated and named by the French chemist ROBIQUET in 1832

Six papers are known by SERTÜRNER on his studies of opium These investigations were published during the years 1805–1817, and five of the original communications can be found reprinted in KROMEKE's book (1925) on the life and work of SERTURNER The two most important papers appeared in the years 1806 and 1817 (SERTURNER 1806, 1817) In the first of these papers, SERTURNER was able to *demonstrate that* the "sleep producing substance" in opium, which he isolated and which was subsequently named *morphine* has basic properties The second of the two studies gives a detailed account of the isolation of morphine from opium, and a description of the toxic effect of the substance in experiments on humans and animals In this connection it is remarkable with what modest means SERTURNER carried out his experiments, and under circumstances which even by the standards of the time were very primitive HANZLIK (1929) writes very pertinently on SERTÜRNER s work. '*It required no munificent grant, no extensive laboratory equipment, no highly organized institute or factory, in fact, it required nothing but the ordinary equipment of a pharmacy and the self-determination of a man*" It may thus be said with greater justice about SERTÜRNER than about most others, that he carried out his experiments "on a corner of the kitchen table"

In addition to the studies by KROMEKE and HANZLIK, the older as well as the more recent literature contains several surveys on SERTURNER and his work on the isolation of morphine in a pure form The studies by HOLM-

of the processes which lead to tolerance to the analgesic effect and certain other effects of these substances

As the title indicates, the present study is concerned with the analgesic effect of morphine and codeine in experiments on tolerant and non tolerant (normal) rats By *analgesia* or *analgesic effect* will be understood here a reduced (or abolished) reaction to a defined painful stimulus following the administration of morphine or a morphine like substance By *analgesic doses* of morphine or a morphine like substance, is understood such doses which give a definable analgesic effect in a definite number of animals (< 100 %) in a given experimental series

The analgesic effect can be determined in the rat with a greater reliability than in the majority of other experimental animals normally employed The rat is also a suitable experimental animal for studying the development of tolerance to the analgesic effect of morphine and codeine This animal is also of such a size, that by sensitive methods of analysis it is possible to determine the concentration of the substances in the brain following the administration of analgesic doses The literature on morphine and morphine-like substances contains numerous examples of experiments performed on animals of different species and employing various methods for measuring analgesia, and these experiments have given exceedingly contradictory results In his experiments, therefore, the author has limited himself to one definite type of experimental animal, namely the rat and hopes that hereby more consistent results have been achieved than would otherwise have been the case using animals of various species for the experiments The analgesic effect of morphine and codeine was also determined by means of two reliable methods which, in the hands of the author, have given comparable and reproduceable results.

I. The analgesic effect of morphine and codeine

A. The localization of the analgesia

The pain-carrying sensitive nerve fibres terminate as free bare filaments in the tissues Together with other sensitive nerve fibres, the pain-carrying fibres enter the spinal cord via the dorsal roots Part of the pain-carrying fibres run as primary conducting pathways in the posterior tracts, while others terminate in the dorsal horns, from which secondary pathways originate (LUNDSGAARD 1964, cf also GORDONOFF 1959) The most important secondary pain-carrying fibres run to the thalamus in the *lateral spinothalamic tract* It is the general view that the fibres in the lateral spinothalamic tract transfer impulses to the *nucleus ventralis posterolateralis*

STEDT & LILJESTRAND (1963) and MØLLER (1966) might be mentioned in particular

Soon after their production in a pure form, morphine and codeine were tested clinically Studies by MAGENDIE (1818) and BARBIER (1834) are among the very first reports of the clinical application of morphine and codeine, respectively Both these authors emphasize the narcotic effect of these substances MAGENDIE likewise emphasizes the analgesic and emetic effect of morphine, as well as its euphoric effect ALLBUTT (1870), on the other hand, appears to be the first to warn against the uncritical use of morphine in the relief of pain and as a sedative drug In this connection, therefore, it is remarkable that almost 50 years previously, COOPER (1823) in an article in *The Lancet* had strongly warned against addiction to opium ("It is a habit which grows upon persons excessively, and ought never to be indulged") The pioneering experimental studies on tolerance to morphine were subsequently followed-up, and are due in particular to authors such as FAUST (1900) and CLOETTA (1903) The first thorough experimental investigations of morphine and other opium alkaloids, however, were carried out by the famous French scientist CLAUDE BERNARD, and were published in 1864 in his *Recherches experimentales sur l'Opium et ses Alcaloïdes*

GUNN (1923) has a valuable review of the older literature on morphine tolerance, while KRUEGER, EDDY & SUMWALT have provided a critical cumulative review of the literature on the pharmacology of the opium alkaloids up to 1940 (cf below) The work of KRUEGER, EDDY & SUMWALT has in part been continued by REYNOLDS & RANDALL (1957) and by SCHAUMANN (1957) As yet, however, no review is available of the literature which has appeared during the last decade

The above mentioned study by CLOETTA is no 3382 in the chronological list of references in the monumental work by KRUEGER, EDDY & SUMWALT, *The Pharmacology of the Opium Alcaloids,* which appeared in two volumes during the years 1941–1943 When KRUEGER, EDDY & SUMWALT ended their work, the number of papers recorded, the majority of which are concerned with morphine, had increased to the three-fold Today, about 25 years later, there is no reliable account of the present extent of the literature on morphine and codeine and the synthetic or semi-synthetic substances with morphine-like effects (by *morphine-like substances* in this study is understood other euphoric analgesics), but the number of published papers has undoubtedly been increasing strongly MILTHERS (1964) is thus no doubt correct in claiming that there are hardly other drugs which have been studied more than morphine and the morphine like substances In spite of the extensive literature on the subject, however, it must be admitted that in general we have so far an extremely imperfect knowledge of the mechanism behind the analgesic and euphoric effect, and only a limited understanding

of the processes which lead to tolerance to the analgesic effect and certain other effects of these substances

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I. The analgesic effect of morphine and codeine

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in the thalamus (WINTER 1965) BAUDOUIN (1951) in a review of the physiology and pathology of pain, has called the lateral spinothalamic tract the "faisceau principal de la douleur" The primary conducting pathways terminate in the medulla oblongata, from which new fibres proceed to the thalamus The thalamus thus appears to receive impulses from all pain-carrying fibres From the thalamus, fibres arise which run as the *tractus thalamocorticalis* to the cerebral cortex (GORDONOFF 1959) *It is thus clear that* in the case of impulses produced by painful stimulation, morphine and similar substances might well effect the transmission right from the bare *filaments in* the peripheral tissue up to the higher centres in the thalamus and cerebral cortex

Formerly, considerable uncertainty has existed as to whether morphine and morphine-like substances influence the excitability and the nerve conduction in the peripheral nervous system KOSTERLITZ & WALLIS (1964) have recently performed experiments with morphine and the peripheral nerve conduction in cats and rabbits They studied nerve conduction both in isolated nerves and in nerves *in situ* Myelinated and unmyelinated nerve *fibres were examined The results showed that morphine had no effect* whatever on nerve conduction, unless very great concentrations were used In the light of these experiments, therefore, there are no grounds for considering that morphine and similar substances block the transmission in the peripheral nervous system On the other hand, it has been known for many years that morphine and morphine-like substances affect transmission in the central nervous system from the spinal column to the cerebral cortex (for a review, see WIKLER 1950) WIKLER maintains that morphine and morphine-like substances depress the after-discharge which develops following the afferent impulses caused by painful stimulation WIKLER sees significance for the analgesic effect in this inhibition Recently published experiments by LIM *et al* (1964) should be referred to in this connection They elicited pain in dogs by intra-arterial administration of bradykinin in micro gram doses as decribed by GUZMAN *et al* (1964) * The animals reacted by vocalization *in the form of a squeak as well as by a general* avoidance reaction The pain reaction could be blocked after the administration of

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*) DEFFENU *et al* (1966) have recently attempted to adapt this method to experiments in rats In these experiments, both codeine, methadone, phenylbutazone aminophenazone, acetylsalicylic acid and phenacetin were used The results suggest that the method can be used both in the determination of the analgesic effect of *morphine and* morphine like substances as well as of that of weaker analgesics The method seems to be rather complicated and is thus hardly suitable for serial experiments (DEFFENU, G, L PEGRASSI & B LUMBACHI The use of bradykinin induced effects in rats as an assay for analgesic drugs *J Pharm Pharmacol* 1966, 18, 135 (a letter to the editor))

both morphine and morphine-like substances, salicylic acid derivatives, paracetamol and phenylbutazone On the basis of carefully performed cross perfusion experiments, the details of which will be omitted here, these authors concluded that morphine and morphine like substances act by blocking the transmission of impulses in the central nervous system, while salicylates, paracetamol and phenylbutazone act by blocking impulse generation at the pain receptors Thus, on the basis of the older as well as the more recent literature, there can hardly be any doubt that the analgesic effect of morphine and morphine like substances is localized to the central nervous system

With regard to the localization of the analgesic effect in the rat, there appear to be only two studies of importance, namely the study by AMSLER from 1921 and the study by CARROLL & LIM from 1960 In experiments on normal rats AMSLER found that following painful stimulation, the animals reacted both by a general avoidance reaction and by squeaks Decerebrate rats (the cerebrum removed by operation) also reacted in this manner following painful stimulation When morphine was administered in small doses it strongly inhibited the pain reaction in the intact animals, while it was without effect on the reaction in the animals operated on AMSLER thus came to the conclusion that the analgesic effect of morphine is localized to some site or other in the cerebrum itself While AMSLER carried out his experiments under rather primitive conditions, CARROLL & LIM were able to perform their experiments using a highly developed technique and equipment Their experiments are described in detail below

CARROLL & LIM (1960) examined the reaction of rats to painful stimulation of the tail The experiments were carried out on animals which had had greater or lesser portions of their central nervous system excluded by means of transection In this manner, an attempt was made to localize the reaction to certain parts of the central nervous system and further experiments were performed following the administration of morphine, codeine and pethidine In the experiments both electrical and mechanical stimulation of the tail were employed It was possible to elicit a graduated reaction in both methods consisting of three stages 1) Movement of the tail and the hind quarters away from the painful stimulus, 2) Squeak response during the stimulation 3) Continued squeak response for a short period after terminating the stimulation A special apparatus made it possible to distinguish between squeak response during and after the stimulation In the electrical stimulation movement of the tail could be triggered off by a voltage of 0 3 Volt on the average A somewhat higher voltage was necessary to elicit the squeak response during and after stimulation, on the average 0 6 and 1 2 Volts Morphine codeine and pethidine affected the three stages in the reaction, however in a reverse sequence The squeak after stimulation was blocked by smaller doses than the doses required to abolish squeak during stimulation,

and even larger doses than these were necessary in order to block the first stage of the reaction (movement of the tail) Transection at a level of the lowest cervical vertebrae blocked the second and third stages but not the first stage Squeak during stimulation was conditional on the medulla oblongata being intact, but could still be elicited after section between the medulla oblongata and the pons Transection in the thalamus region blocked the third stage of the reaction while transection above the thalamus left the pain reaction practically unaffected

These experiments on rats thus provide an experimental basis for the assumption that morphine and morphine-like substances first block that *part of the pain reaction which is localized at the highest level in the central* nervous system (squeak after stimulation) It is remarkable that it should be just this part of the reaction which required stimulation with a greater intensity than was the case for the other two stages

The results also suggest that the analgesic effect is localized to a particular degree in the diencephalon, and there particularly in the thalamus Morphine, however, must also be assumed to affect the pain reaction all the way through the central nervous system (cf also IRWIN *et al* (1951) page 21)

B. *Methods for determining analgesia*

Although painful stimulation was used in experiments on animals as early as last century (cf BEECHER 1957), EDDY (1928) and HAFFNER (1929) appear to be the first to have described and worked out methods in detail for determining analgesia in animal experiments Both these authors elicited pain by mechanical stimulation HAFFNER used clips which could be fastened on the tail of mice, or on the ears of guinea pigs and rats HAFFNER's method and its numerous modifications have been used in particular in experiments on mice EDDY constructed a special press unit, whereby a variable pressure could be exerted on the distal part of the tail in the cat In a subsequent publication, EDDY (1932) has described his procedure in detail

During the 35 years since then, a large number of methods and modifications of methods have been described for determining analgesia in animal experiments In a detailed review, BEECHER (1957) provides a critical evaluation of these methods of measurement An excellent review of methods for determining analgesia in animal experiments has also been provided recently by WINTER (1965) The following will therefore be restricted to a short account and discussion of the most important methods and modifications of methods which have been used for determining analgesia in experiments with rats as experimental animals in particular The methods are grouped according to the type of stimulation used to elicit pain

1) Mechanical stimulation

FRIEND & HARRIS (1948) used a forceps whereby pressure could be exerted on the tail of the rat. The rats reacted with a squeak response when the pressure was raised to a certain value Without administration of analgesics the rats reacted fairly constantly with a squeak response when the diameter of the tail was compressed to 8 1–8 3 mm between the arms of the forceps Following subcutaneous injection of 5 mg/kg morphine, an increased degree of compression was necessary to elicit pain Quantitative studies on the dose-effect relationship were not made

GREEN, YOUNG & GODFREY (1951) elicited pain by exerting pressure on the tip of the tail The pressure was exerted through the medium of a syringe piston placed in a vertical syringe so that the free end of the piston rested on the tip of the tail A pump fills the cylinder slowly and at a uniform rate with a mixture of paraffin oil and kerosine so that the piston is pressed out of the syringe and down onto the tip of the tail A T-tube connects the pump with the syringe and a mercury manometer GREEN *et al.* read off the pressure in the system (cm Hg) both when the rats were struggling to release the tail and when they reacted with a squeak response The rats first reacted with the tail response and then with the squeak response if the pressure continued to be increased A linear rise in the threshold values was found (semilogarithmic representation) 30 minutes after administration of morphine in increasing amounts (2–8 mg/kg subcutaneously)

2) Electrical stimulation

MACHT & MACHT (1940) were the first to describe a method in which electrical stimulation was used to elicit pain in experiments on rats They implanted two electrodes in the skin of the scrotum The electrodes were connected with an induction apparatus The rats reacted with a squeak response when the voltage was increased over a certain threshold, the pain threshold This threshold was found to be very constant in the individual animal The results are difficult to interpret

LUCKNER & MAGUN (1951) implanted two electrodes in the upper part of the tail Shocks were given of rectangular wave form of 10 milliseconds in duration and with a frequency of 2 per second from an impulse generator The rats reacted with a squeak response The normal pain threshold was between 0 55 and 0 9 mA The pain threshold was very constant in the individual animal The analgesic effect of morphine and pethidine, administered in increasing doses, was expressed by the percentage rise in the pain threshold

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the upper part of the tail Rectangular impulses of 20 milliseconds in duration and with frequency of 1 per second were sent through the electrodes from an impulse generator The voltage was 3 Volts and the current strength 0 2 mA About 80 % of the rats reacted by means of a squeak response to the first, second, third or fourth shock By far the majority of the animals reacted to one of the first two stimuli The remaining 20 % of the animals, which did not react to the first four stimuli, were not used in the experiments on analgesia The reactive rats (approx 80 %) were stimulated twice at an interval of 30–45 minutes In the individual animal, the number of stimuli required to elicit the squeak response was found to vary with at most ± 2 stimuli in the two repeated experiments In experiments with analgesics the reaction of the rats was tested 15–20 minutes before and 30 minutes after the administration If the reaction of the animals after the injection exceeded the threshold from before the injection by 3 stimuli or more, this was regarded as a positive analgesic reaction The results of the experiments were recorded in a bilogarithmic system, with the percentage number of animals showing a positive analgesic reaction as ordinate and the dose as abscissa In the case of morphine (2 5, 5 0 and 10 mg/kg intraperitoneally), codeine (10, 20 and 40 mg/kg intraperitoneally) and normorphine (10 20 and 40 mg/kg intraperitoneally), the three points for each substance were found to lie on a straight line

COLLINS *et al* (1964) implanted one electrode in the rectum and the other one in the tail Rectangular impulses of 10 milliseconds duration and with a frequency of 50 per second were sent through the electrodes from an impulse generator The animals reacted with a squeak response The voltage was increased by 1 Volt per second, and the analgesic effect was expressed by the rise in pain threshold in Volts The animals received subcutaneous injections of morphine and codeine, respectively, in 6 different doses The results were depicted in a coordinate system with the dose as the abscissa and the rise in the pain threshold in Volts as the ordinate In the case of codeine, all 6 points were found to lie on a straight line, but this was not the case with morphine If the results had been depicted in a logarithmic system, however, there is no doubt that also in the morphine experiment all 6 points would have lain on a straight line

3) Thermal stimulation

a) Radiant heat

WOLFF, HARDY & GOODELL in 1940 described a method based on radiant heat to produce pain, often used previously for determination of analgesia in experiments on human subjects D'AMOUR & SMITH (1941) modified

this technique for experiments on rats They irradiated the tail tip with heat from a light source of a definite strength D'AMOUR & SMITH performed about 10,000 separate experiments using several hundred rats, and found that in more than 99 % of all experiments the rats reacted in the course of 6 seconds by flicking or moving the tail away from the light source ('tail flick") 'Complete analgesia" was defined as a complete absence of reaction to the painful stimulus ('the animal makes no movement of the tail whatever though it is being burned to a crisp") When morphine and codeine were administered in increasing doses this increased the percentage of animals showing "complete analgesia" The method of D'AMOUR & SMITH, together with its numerous modifications, only a few of which will be mentioned below, have undoubtedly been those procedures most employed during the last 25 years for determination of analgesia in experiments on rats In this connection it may be mentioned that IRWIN *et al* (1951) were able to elicit the "tail flick" reaction in spinal rats (spinal column sectioned between the 5th and the 8th thoracic vertebrae) as early as 5 minutes after operation The influence of morphine and methadone on the reaction was found to be considerably less in the animals operated on than in the intact animals

DAVIES RAVENTOS & WALPOLE (1946) used a modification of the method of D'AMOUR & SMITH A reaction time of 15 seconds following administration of analgesics was defined as "complete analgesia" THORP (1946) and HOUGS OLSEN (1949) have used the same principle as D'AMOUR & SMITH for determining analgesia in experiments on rats WINTER & FLATAKER (1950) used the method of D AMOUR & SMITH in experiments with morphine and methadone The difference between the reaction time of the animals before and after the injection, measured in seconds was multiplied by the number of minutes which had elapsed since the injection until the analgesic effect was determined Both the intensity and the duration of the analgesic effect thus enter into the product, the unit of which was designated minute seconds"

ERCOLI & LEWIS (1945) shaved a region on the back of rats and irradiated it with light of a definite intensity The rats normally [illegible]

[illegible] of all experiments the rats reacted by contraction in the skin in the course of 6 seconds When injected subcutaneously 4 *mg/kg* was the least *dose* of morphine which prolonged the reaction time beyond 6 seconds, while 20 *mg/kg* produced "complete analgesia" (reaction time of 15 seconds)

CAHEN EPSTEIN & KREMENTZ (1948) modified the method of ERCOLI & LEWIS They used irradiation of a definite strength for a fixed period, usually

8 seconds After administration of morphine, codeine or other analgesics, a linear relationship was found between the logarithm of the dose administered and the probit value for the number of rats not reacting to the painful stimulus ("probit of rats protected from painful stimulus")

b) Conducted heat

WOOLFE & MACDONALD (1944) described a method for determining analgesia in experiments on mice The animals were placed in a glass cylinder on a zinc plate which was heated to 55–70° The mice reacted to the heat by standing on their hind legs and licking their forepaws or wriggling with them EDDY and co-workers (EDDY, TOUCHBERRY & LIEBERMAN 1950, EDDY & LEIMBACH 1953) have improved the technique of WOOLFE & MACDONALD considerably The technique used by EDDY involves placing the mice in a cylindrical glass container, the base of which is made of a copper plate This copper plate is at the same time the "roof" in a circular, flat-bottomed vessel containing 1200 ml of an equal mixture of ethyl formate and acetone The whole apparatus stands on a hot plate so that the mixture boils EDDY and co-workers found that the boiling point was constant between 55 and 55 5° Vapour from the boiling mixture is conducted to a water-cooler so that the vapour condenses and is led back to the supply This technique is often called EDDY's hot plate

DANDIYA & MENON (1963), COCHIN & KORNETSKY (1964) and JÓHANNESSON & WOODS (1964) have used EDDY's hot plate for the determination of analgesia in experiments on rats JÓHANNESSON & WOODS (1964) have given a detailed description of their modification of the method It has been employed and examined in further detail by the author of the present study, in unpublished experiments (JÓHANNESSON 1964), the results of which will be described in part below The experiments were carried out by two persons The one placed the rats on all four paws on the plate and then placed a "flat hand" over the upper opening of the cylinder The other person read off the reaction time of the animals on a stop watch The rats reacted either by licking their paws or by jumping out of the cylinder sideways from the hand The great majority of the rats, however, reacted in the way first described When analgesics were not administered, all rats reacted from 3–9 seconds after they were placed on the plate In experiments where the reaction time was measured every 30 minutes in the course of 2½ hours, a mean reaction time was found of 5 7 seconds ± 1 6 (standard deviation) In the 6 measurements, none of the rats showed individual differences greater than 3 seconds Following the administration of analgesics a prolongation of the reaction time in the individual animal by more than 3 seconds over the normal value was regarded as an analgesic reaction The

pain threshold remained unchanged by administering the solvent (demineralized water) The results of the experiments with morphine, both subcutaneously and intraperitoneally (s c 1 25, 2 5 and 5 0 mg/kg, i p 2 5 5 0 and 10 mg/kg), and codeine (s c 20, 40 and 60 mg/kg, i p 7 5, 15 and 30 mg/kg) were depicted in a bilogarithmic system with the percentage number of animals showing analgesic reaction as ordinate and the dose as abscissa In each set of experiments the three points were found to lie on a straight line

Another form of hot plate was described by PORSZASZ, TARDOS HERR & NYIRI (1953) These investigators used a circular flat bottomed tin kettle filled with water The kettle was heated to a constant temperature of 56° The rats were placed on the surface of the kettle in a glass cylinder and the reaction time read off The rats reacted by licking their paws Without the administration of analgesics the reaction time was found to be rather constant at 8–12 seconds In this connection it may be mentioned that the normal pain reaction (licking of paws) is not seen in rats where the cerebral cortex has been removed (cf HERR, TARDOS & PORSZASZ 1953)

JACKSON (1952) described a method in which tail flick" in the rat was elicited by conducted heat The method is only mentioned here for the sake of completeness as it is apparently more complicated than the method of D AMOUR & SMITH and has hardly any advantages over the latter

4) Comparison of methods

If two or more methods for measuring analgesia are to be *compared with regard* to sensitivity, the same investigator (or investigators) should *work with these methods* concurrently However, *the use of* two methods for determ ning analgesia in rats has apparently been *employed* by few, and no one appears to have used three methods.

HERR, TARDOS & PÓRSZÁSZ (1953) used the method of D AMOUR & SMITH (1941) and their own form of hot plate (see above) for determining analgesia in experiments on rats [illegible]
of morphine were requ [illegible]
method than by the met [illegible]
morphine and methador [illegible]
les later They used the [illegible] with mechanical stimulation of the tail (see page 19) as well as the method of D AMOUR & SMITH The sensitivity to morphine and methadone was almost the same in both methods and ED_{50} for morphine was 2 3 times *greater than* for methadone JOHANNESSON & SCHOU (1963 a, b) and JOHANNESSON & WOODS (1964) determined the analgesic effect of morphine and codeine *30* minutes after intraperitoneal injection They used respectively, electrical stimulation *of the* tail and

a modification of EDDY's hot plate (see pages 20 and 22) ED_{50} for morphine and codeine, respectively, was about the same in both experiments, which however, were not performed in rats of the same strain DANDIYA & MENON (1963) used EDDY's hot plate together with a method based on mechanical stimulation of the tail Following intraperitoneal administration of 3 mg/kg morphine, 14 rats out of 20 showed an analgesic reaction by EDDY's technique, and 12 out of 20 by the other method

It is remarkable that in their experiments, HERR, TARDOS & PORSZASZ found that the "tail-flick" reaction is a more sensitive criterion than paw licking (their own hot plate method) Methods based on electrical or mechanical stimulation of the tail, however, do not appear to be more sensitive than EDDY's hot plate method (cf JOHANNESSON *et al* and DANDIYA & MENON above) As mentioned, GREEN *et al* (1951) found good agreement between mechanical stimulation of the tail, in which the pain reaction is avoidance response and a squeak response, and the "tail-flick" reaction It is thus difficult to compare the results of various authors As already mentioned, *a useful comparison of sensitivity of the various methods for measuring* analgesia in experiments on rats can only be obtained if such experiments are carried out by a single experimentor or working group and are performed in a definite rat strain

5) Choice of method

No matter which method is used for measuring analgesia there should exist within a given dosage range a reasonable relationship between the dose and the effect, in order to obtain quantitatively comparable results All the methods mentioned appear to satisfy this demand with the exception of two or three of the oldest methods which are more qualitative than quantitative The method used should moreover be sensitive, do least harm to the animal and be easy to perform It is also an absolute advantage if the technique used does not require expensive or complicated apparatus

As far as is possible on the basis of the few results available, the sensitivity of the methods has been discussed above Electrical stimulation which requires implantation of electrodes, and mechanical stimulation of the tail or other parts of the body, can easily result in lesions to the animals This is unfortunate if the animals are to be used several times as for example when the development of tolerance is to be investigated *Stimulation by heat* whether by radiation or by conduction, must be considered a relatively mild procedure which does not require a complicated apparatus and is technically easy to perform The choice will thus primarily *lie between those methods* where heat is used as a stimulus

Paw-licking presupposes a complicated motor integration in the brain

The avoidance reaction of the tail ("tail-flick"), on the other hand, is a reflex movement, the reflex center of which is localized in the spinal column It may therefore be assumed that inhibited paw-licking, rather than inhibited "tail-flick", indicates that the conscious reaction of the animal to pain has been reduced Thus, in the present author's opinion, EDDY's *hot plate is to* be preferred to the method of D'AMOUR & SMITH In his latest experiments, where the analgesic effect of morphine and codeine was studied during the development of tolerance the present author has therefore used only the modification of EDDY's hot plate This method has given particularly reproduceable results in the author's hands With one exception (cf JOHANNESSON, ROGERS FOUTS & WOODS 1965 b) the degree of the analgesic effect has been expressed by the percentage number of animals showing an analgesic reaction according to the criteria used In the study by JÓHANNESSON *et al* (1965 b) mentioned above, particular circumstances held, as these experiments aimed at a precise timing of the development *of tolerance* to morphine and codeine In that study, therefore, the analgesic effect of morphine and codeine during the development of tolerance was expressed both by the number of animals showing the analgesic reaction, and by the mean reaction time of the animals in seconds (cf page 41)

C. The time course of the analgesic effect

The *first* study of the time course of the analgesia is apparently due to D AMOUR & SMITH (1941) *These investigators* determined the analgesic effect of morphine and codeine administered by *intraperitoneal injection*, 30 120 and 150 minutes after the administration of the drugs The effect was a maximum 30 minutes after the injection, while the duration was to some extent dependent on the magnitude of the dose Other authors have subsequently performed similar investigations with different methods of measurement

ERCOLI & LEWIS (1945) thus determined the analgesic effect of morphine and codeine 15, 30, 45, 60 120 and 180 minutes following intraperitoneal and subcutaneous administration The effect commenced 15–30 minutes after the injection As far as appears from the results, equianalgesic, average doses ("average analgesic doses") *of morphine and codeine administered* subcutaneously and intraperitoneally, had their greatest effect 30–60 minutes after the injection The effect lasted from 60–120 minutes Following the administration of large doses the intensity of the analgesia was found to be "complete" (see page 21) as early as 30 minutes after the injection and the effect was maintained for about 200 minutes These drugs had little effect when given *per os* Administered intravenously, morphine and codeine had a brief and potent but often uncertain *effect* In agreement with this,

a modification of EDDY's hot plate (see pages 20 and 22) ED_{50} for morphine and codeine, respectively, was about the same in both experiments, which however, were not performed in rats of the same strain DANDIYA & MENON (1963) used EDDY's hot plate together with a method based on mechanical stimulation of the tail Following intraperitoneal administration of 3 mg/kg morphine, 14 rats out of 20 showed an analgesic reaction by EDDY's technique, and 12 out of 20 by the other method

It is remarkable that in their experiments, HERR, TARDOS & PORSZASZ found that the "tail-flick" reaction is a more sensitive criterion than paw licking (their own hot plate method) Methods based on electrical or mechanical stimulation of the tail, however, do not appear to be more sensitive than EDDY's hot plate method (cf JOHANNESSON *et al* and DANDIYA & MENON above) As mentioned, GREEN *et al* (1951) found good agreement between mechanical stimulation of the tail, in which the pain reaction is avoidance response and a squeak response, and the "tail-flick" reaction It is thus difficult to compare the results of various authors As already mentioned a useful comparison of sensitivity of the various methods for measuring analgesia in experiments on rats can only be obtained if such experiments are carried out by a single experimentor or working group, and are performed in a definite rat strain

5) Choice of method

No matter which method is used for measuring analgesia, there should exist within a given dosage range a reasonable relationship between the dose and the effect, in order to obtain quantitatively comparable results All the methods mentioned appear to satisfy this demand with the exception of two or three of the oldest methods, which are more qualitative than quantitative The method used should moreover be sensitive, do least harm to the animal and be easy to perform It is also an absolute advantage if the technique used does not require expensive or complicated apparatus

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authors on the interval elapsing before the maximum effect develops, there is considerably greater uncertainty with respect to the duration of the effect The results suggest that the analgesic effect of the morphine and codeine following subcutaneous or intraperitoneal administration, can be measured for up to about 1½–3½ hours, and that the duration is to some extent dependent on the size of the dose In this connection it is of some interest that JOHANNESSON *et al* (1965 b) and JÓHANNESSON & WOODS (1965) found that after subcutaneous injection (cf above), the effect of codeine lasted longer than that of morphine The amounts administered had the same analgesic effect 30 and 60 minutes after the injection, but already after 90 minutes the analgesic effect of codeine was significantly greater than that of morphine (JÓHANNESSON & WOODS 1965) In one of their diagrams (fig 3), ERCOLI & LEWIS (1945) have indicated that 70 mg/kg codeine administered subcutaneously had a considerably longer lasting analgesic effect than the same dose of codeine administered intraperitoneally These findings, however, were not commented on further In line with this, JOHANNESSON (1965) found that lethal doses of codeine injected intraperitoneally had their effect 1–2 hours earlier than lethal doses of codeine injected subcutaneously The prolonged analgesic effect and the slow onset of the lethal effect after subcutaneous administration of codeine can reasonably be ascribed to the fact that codeine is absorbed more slowly from the subcutaneous connective tissue than from the abdominal cavity

The analgesic effect of morphine and codeine administered by intravenous injection apparently develops in the course of only a few minutes and is of very short duration

D. Influence of the route of injection on the intensity of analgesia

In experiments on analgesia *in rats*, morphine and codeine are usually administered either subcutaneously or by *intraperitoneal* injection It is of interest, therefore, to attempt to evaluate how these two most important routes of injection influence the *intensity* of the analgesia On the present basis this can best be done by comparing the analgesic effect of morphine and codeine as found by the individual authors under the given experimental circumstances.

In experiments with *subcutaneous administration* of morphine and codeine, ERCOLI & LEWIS (1945) found as "average analgesic dose" for morphine, 6 0 mg/kg, and for codeine, 42 mg/kg CAHEN *et al* (1948) found that the "average effective dose" for morphine was 3 0 mg/kg and for codeine 24 mg/kg COLLINS *et al* (1964) found that 5 mg/kg morphine had approximately the same analgesic effect as 30 mg/kg codeine JÓHANNESSON & WOODS

JACKSON (1952) *found that the analgesic effect of morphine administered* intravenously could usually be recorded within 5 minutes of the injection However, the effect was often found to be a maximum as early as 1 minute *after the administration, and it was of very short duration*

THORP (1946) found that morphine injected subcutaneously had its maximum analgesic effect 30–60 minutes after The analgesia lasted for 180 *minutes or more* LUCKNER & MAGUN *(1951) found the greatest effect 40* minutes after the subcutaneous administration of morphine The effect was almost off 120 minutes after WINTER & FLATAKER (1953), in agreement *with this, found that the analgesic effect of morphine was a maximum 30–60* minutes after subcutaneous injection The effect was decreasing after 120 minutes

CAHEN *et al* (1948) *found that when administered subcutaneously,* equianalgesic, average doses ("average effective doses") of morphine and codeine had their greatest effect after 30 minutes The effect lasted for *about 200 minutes, according to the criteria used* COLLINS *et al (1964)* administered morphine and codeine subcutaneously and determined the analgesic effect at intervals of 30 minutes over a period of 180 minutes *The analgesic effect was greatest 30–60 minutes after the injection and it* had not disappeared by the end of the period of observation, except in the case of the least dose of codeine JOHANNESSON & WOODS (1964) found *that the analgesic effect of morphine (given subcutaneously and intraperi-*toneally) and codeine (given intraperitoneally) was greater after 30 minutes than after 60 minutes The effect of codeine administered subcutaneously *on the other hand, was about the same after 30 and 60 minutes* JÓHANNESSON, ROGERS, FOUTS & WOODS (1965 b) and JÓHANNESSON & WOODS (1965) likewise administered morphine by subcutaneous and intraperitoneal injec*tion, and codeine by subcutaneous injection,* after which the analgesic effect was determined at intervals of 30 minutes during a period of 90–150 minutes The results showed regularly that the analgesic effect was greatest 30 mi*nutes after the administration* The analgesic effect of morphine was off 120 minutes after, while after subcutaneous injection, codeine still showed a measurable effect 150 minutes after the injection

The results discussed show that after both subcutaneous and intraperitoneal administration of morphine and codeine, the intensity of the analgesia is greater 30–60 minutes after than at any other period The experimental results, however, rather seem to favour a maximum effect after 30 minutes than after 60 minutes The findings of ERCOLI & LEWIS (1945) suggest than when morphine and codeine are administered subcutaneously and intraperitoneally, there is hardly any measureable analgesic effect until 15 minutes after the injection, but further experiments are necessary to elucidate this Thus, while there is good agreement between the reports by the various

process (cf WOODS 1954 and OTOBE 1960) The strong analgesic effect of morphine after subcutaneous injection therefore, can reasonably be explained by the fact that the substance is absorbed from the peripheral tissues without passing through the liver In mice, morphine is also found to have a greater effect after subcutaneous injection than after intraperitoneal injection (BIANCHI & FRANCESCHINI 1954)

In rats, codeine is conjugated to glucuronic acid in the liver to a far lesser extent than morphine (about 10 times less, YEH 1964, personal communication) Codeine must therefore be regarded as mainly present in "free" or active form after passage through the liver, a state which must be highly advantageous for the biological effect of this substance This can explain why codeine, in contrast to morphine, has a stronger effect after intraperitoneal administration than after subcutaneous administration

E. Simultaneous administration of morphine and codeine

Morphine and codeine can increase each other's inhibitory effect on respiration in mice (RIKL 1928) The synergistic effect on the respiration was characterized as additive Apart from his own studies, the present author has not found any reports in the available literature on analgesic experiments with rats or mice in which morphine and codeine were administered simultaneously Nor does this appear to have been the subject of thorough investigations, since KRUEGER, EDDY & SUMWALT (1943) wrote 'Opiates mixed among themselves have been very inadequately studied either in the laboratory or in the clinic, despite their wide use"

JÓHANNESSON (1965) injected morphine (2 5 or 5 0 mg/kg) and codeine (3 5–60 mg/kg) subcutaneously into rats with the least possible delay between the injections The substances were not administered at the same site The analgesic effect was determined 30 minutes later The results showed that morphine and codeine always increased each other's analgesic effect, and that the synergism might well be more than a simple addition of the effects of the two substances It was also found that morphine and codeine always increased each other's lethal effect

JÓHANNESSON & WOODS (1965) administered morphine and codeine by subcutaneous injection in doses which had equianalgesic effect 30 and 60 minutes after the injection Morphine or codeine administered in doses which were 17 times less were without measureable analgesic affect It was found, however, that the simultaneous administration of the smaller (non analgesic) dose of codeine and the greater (analgesic) dose of morphine resulted in a significant increase in, but hardly a prolongation of the analgesic effect of the morphine After administration of the larger dose of codeine and the smaller non analgesic dose of morphine, the analgesic effect of codeine was

(1964) on the other hand, found that the ED_{50} for morphine and codeine was, respectively, 4 0 and 45 mg/kg

In *intraperitoneal administration* of morphine and codeine, D'AMOUR & SMITH (1941) found that 10 mg/kg morphine had a slightly weaker effect than 24 mg/kg codeine DAVIES *et al* (1946) found that 10 mg/kg morphine gave the same degree of analgesia as 30 mg/kg codeine ERCOLI & LEWIS (1945) found that 10 mg/kg morphine and 49 mg/kg codeine were equianalgesic doses JOHANNESSON & SCHOU (1963 a) found an ED_{50} for morphine of 6 5 mg/kg and for codeine of 21 mg/kg In a subsequent investigation the corresponding figures found were, respectively, 7 0 and 20 mg/kg (JÓHANNESSON & WOODS 1964)

The experimental results mentioned thus show that morphine has an effect which is about 6–11 times stronger than that of codeine in subcutaneous administration, while in the case of intraperitoneal injection it is only about 2 5–5 times as potent

ERCOLI & LEWIS (1945) and JÓHANNESSON & WOODS (1964) found in agreement that morphine injected subcutaneously had almost twice as potent an effect as morphine administered by intraperitoneal injection HERKEN *et al* (1959) and WINTER & FLATAKER (1965 a), in line with this, have also found that the analgesic effect of morphine after subcutaneous injection is twice as strong as after intraperitoneal administration In the case of codeine, however, JOHANNESSON & WOODS (1964) found that after intraperitoneal injection it had twice as strong an effect as after subcutaneous injection while ERCOLI & LEWIS (1945), on the other hand did not find any difference JOHANNESSON (1965), in a subsequent experimental series has confirmed the earlier demonstration of the strength of the codeine effect following subcutaneous and intraperitoneal injection The relatively strong effect of morphine administered subcutaneously, therefore, can partly explain why the ratio between the analgesic effect of morphine and codeine is greater following subcutaneous administration than following intraperitoneal administration As mentioned, it was demonstrated by JÓHANNESSON & WOODS (1964) and JÓHANNESSON (1965) that codeine administered intraperitoneally has a greater analgesic effect than codeine administered subcutaneously This will also help to explain why the ratio between the strength of the effect of morphine and codeine is greater following subcutaneous administration than following intraperitoneal administration

When administered subcutaneously, morphine is absorbed directly from the subcutaneous connective tissue into the circulation, after which it is distributed throughout the body On the other hand morphine administered intraperitoneally will pass through the liver via the portal circulation In the liver, morphine is conjugated to glucuronic acid (cf STROMINGER *et al* 1954 and RINK *et al* 1956), and the conjugation is a pronounced inactivation

PÆRREGAARD (1957) modified the method to determine morphine in urine, and MILTHERS (1958, 1959) used it to determine morphine in blood and tissue *homogenates* PÆRREGAARD isolated morphine by means of paper chromatography, while MILTHERS omitted this purification procedure, as tissue homogenates and blood from rats were not found to contain substances which interfered with the morphine analyses JOHANNESSON (1962 b), however, has demonstrated clearly that such interfering substances can be found in the brain when morphine is administered to rats together with another substance (neostigmine) In their modification of the method, therefore, JOHANNESSON (1963) and JÓHANNESSON & MILTHERS (1964) have introduced paper chromatography as a standard procedure in the determination of morphine in the brain The last named authors increased the sensitivity of the method considerably, so that amounts as low as 0 2 µg could be determined in pure solutions In recovery experiments, it was possible to determine amounts down to 0 4 µg/1–2 g brain, with reasonable certainty In the case of amounts from 0 6–1 0 µg/1–2 g brain, the recovery was 90–95% with an uncertainty of ±3–8% On the other hand the recovery percentage for 0 4 µg/1–2 g brain was constantly above 100% (103–110%), which was due to technical difficulties in evaluating the polarograms

As mentioned, paper chromatography is included in the technique It must thus be characterized as both exceedingly specific and rather sensitive On the other hand, the method is relatively time-consuming Normorphine and nalorphine can be determined by polarography in the same way as morphine These substances, however, can be separated with certainty from morphine (MILTHERS 1961, JÓHANNESSON 1963) JÓHANNESSON & SCHOU (1963 a, b) have employed the polarographic method for determining morphine and normorphine in the brain of rats after administration of these substances in analgesic doses

SZERB, MACLEOD, MOYA & MCCURDY (1957) described a *colorimetric method* for the determination of morphine The method is based on the principle that Folin-Ciocolteu's reagent (phosphotungstic- phosphomolybdic acid), in the presence of phenolic compounds such as morphine, forms a blue colour The method includes precipitation with benzene and the isolation of morphine by means of adsorption on to ion-exchange resins About 80% of the morphine added to biological materials is recovered The least measureable concentrations are about 3 µg/ml blood and /g tissue, and 0 6 µg/ml plasma SZERB & MCCURDY (1956) used this method to determine morphine in the brain and blood of rats.

KUPFERBERG, BURKHALTER & WAY (1964) have recently worked out a *spectrofluorometric method* for determining morphine The method is based on the principle that morphine is converted to pseudomorphine (oxidimorphine), which can be determined by spectrofluorometry Morphine has a

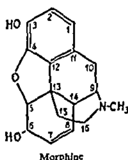
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The synergistic effect of morphine and codeine deserves a thorough investigation with respect to the question whether it is additive or potentiated In particular, it will be desirable to have this examined in human subjects by means of a thorough clinical pharmacological study, as emphasized by JOHANNESSON & WOODS (1965)

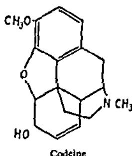
II. Morphine and codeine in the brain and the analgesic effect

A. *Methods for determining morphine and codeine in the brain*

PÆRREGAARD (1958 b) and MILTHERS (1964), in their theses in Danish from the Department of Pharmacology at the University of Copenhagen, have provided reviews of methods for determining morphine in biological materials In this connection they have given detailed accounts of the extraction and purification procedures generally employed WAY & ADLER (1962) have also published a review of the subject in English The present study will therefore only briefly mention those methods which are important in the quantitative determination of such microgram quantities of morphine and codeine as are found in the brain of experimental animals following the administration of these substances



Morphine



Codeine

1) Methods for determining morphine

BAGGESGAARD RASMUSSEN, HAHN & ILVER (1945) worked out a *polarographic method* to determine morphine in the presence of other opium alkaloids Morphine itself cannot be determined by polarography, but after treatment with nitrous acid, a nitroderivative is formed (cf BAGGESGAARD RASMUSSEN & BOLL 1958 and LUND 1958) which can be determined polarographically

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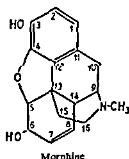
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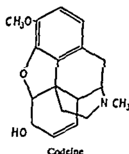
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weak fluorescence, while pseudomorphine has a powerful fluorescence The least measurable amounts are reported to be 0 1 μg These authors used the method *to determine morphine in the blood of rabbits following* intramuscular and intravenous administration When the method is modified it can be used to determine normorphine and nalorphine, for example As far as is known, the method has not been used to determine morphine in the brain of rats or other experimental animals following administration of the drug in analgesic doses

It should be emphasized here that substances producing fluorescence can be demonstrated in tissue sections, just as it is possible to obtain an impression of the amounts present It should therefore also be possible that once morphine had been converted to a fluorescent substance, it could also be *demonstrated in the tissues by means of fluorescence microscopy*

Numerous recent studies are based on *radioactive measurements* of morphine, labelled with radioactive carbon (^{14}C) or tritium (^{3}H) RAPOPORT, LOVELL & TOLBERT (1951) and ANDERSEN & WOODS (1959) have described procedures *for the preparation of radioactive morphine labelled with* ^{14}C at the N-methyl group, here designated as morphine-N-$^{14}CH_3$ MARCH & ELLIOTT (1952, 1954) were among the first to use morphine-N-$^{14}CH_3$ in experiments on rats

ADLER, ELLIOTT & GEORGE (1957) used radioactive technique to study the fate of morphine in the rat Morphine was isolated by paper chromato graphy, *after which it was converted to crystalline dinitrophenylmorphine*-N-$^{14}CH_3$ before the radioactivity was measured The method is thus exceedingly specific, and the sensitivity was satisfactory, although it appears from the results presented that the uncertainty of the method is very considerable

MULÉ & WOODS (1962) and MULÉ, WOODS & MELLETT (1962) used morphine-N-$^{14}CH_3$ in experiments on dogs JOHANNESSON & WOODS (1964) with a few modifications, have used the same technique in experiments on rats *The measurements were based on fluid scintillation technique* The lowest measureable amounts were around 5 ng Increasing amounts of morphine (10–1500 ng) gave a linear rise in the intensity of the radioactivity *measured* ("counts per minute") On addition of morphine to homogenates of rat brains, the recovery was about 93% ± 2% (limits of error, P 0 95)

A critical question in radioactive measurements on biological material is whether the radioactivity measured is localized in the molecule of the substance whose amount it is desired to determine Morphine N-$^{14}CH_3$ can undergo N demethylation in the organism (cf chapter IV A) If N demethylation of morphine-N-$^{14}CH_3$ takes place to a great extent, there is no guarantee that the *radioactivity measured* corresponds to authentic morphine MULÉ & WOODS (1962) and MULÉ WOODS & MELLETT (1962) examined whether normorphine or other metabolites were present in the brain following the

administration of radioactive morphine By collecting homogenates of different parts of the brain in dogs which had been killed after having received one or three subcutaneous injections of 2 mg/kg morphine N-$^{14}CH_3$ sufficient material was obtained to be able by means of paper chromatography to demonstrate with reasonable certainty normorphine or possibly other conversion products Paper chromatography provided no evidence of the presence of normorphine, and all the radioactivity was collected at a spot which had the same relative front as authentic morphine The results strongly suggested therefore that the radioactivity measured in the brain was due exclusively to the unconverted morphine molecule Such experiments have not been carried out in the rat It should therefore be emphasized that MILTHERS (1962) even after the administration of very large doses of morphine (100 mg/kg i v) to rats found only very small amounts of morphine in the brain 15 minutes after the administration (morphine concentration 6 2 µg g normorphine concentration < 0.4 µg/g) It is therefore highly probable that following the administration of morphine N-$^{14}CH_3$ in analgesic doses to rats almost the entire radioactivity measured in the brain is due to the unconverted molecule

MISRA & WOODS (1960) described a procedure for the preparation of tritium labelled morphine MISRA, MULÉ & WOODS (1961) used tritium labelled morphine to investigate the conversion of morphine to normorphine in the rat The substances were isolated in the urine by means of paper chromatography As far as the present author is aware tritium labelled morphine has not been used in experiments to determine morphine in the brain Tritium labelled morphine and morphine derivatives can be prepared with a high specific activity As a result, both great sensitivity and specificity can be obtained (YEH personal communication 1964)

2) *Methods for determining codeine*

WOODS *et al* (1954) elaborated a method for the colorimetric determination of morphine in biological materials, which WOODS *et al* (1956) subsequently modified for the determination of codeine in experiments on dogs and monkeys The method is based on the principle that methyl orange in combination with morphine codeine and many other amines, forms a coloured compound which is easily extracted by means of organic solvents The minimum concentration of codeine which could be determined in plasma was 0 5 µg ml with a recovery percentage of 120 ± 20 %.

The other methods [illegible]

based on me[illegible]

procedure for [illegible] of radioactive codeine labelled with ^{14}C in the methoxy group designated here as codeine-O-$^{14}CH_3$ ADLER & LATHAM

weak fluorescence, while pseudomorphine has a powerful fluorescence The least measurable amounts are reported to be 0 1 μg These authors used the method to determine morphine in the blood of rabbits following intramuscular and intravenous administration When the method is modified, it can be used to determine normorphine and nalorphine, for example As far as is known, the method has not been used to determine morphine in the brain of rats or other experimental animals following administration of the drug in analgesic doses

It should be emphasized here that substances producing fluorescence can be demonstrated in tissue sections, just as it is possible to obtain an impression of the amounts present It should therefore also be possible that once morphine had been converted to a fluorescent substance, it could also be demonstrated in the tissues by means of fluorescence microscopy

Numerous recent studies are based on *radioactive measurements* of morphine, labelled with radioactive carbon (^{14}C) or tritium (^{3}H) RAPOPORT, LOVELL & TOLBERT (1951) and ANDERSEN & WOODS (1959) have described procedures for the preparation of radioactive morphine labelled with ^{14}C at the N-methyl group, here designated as morphine-N-$^{14}CH_3$ MARCH & ELLIOTT (1952, 1954) were among the first to use morphine-N-$^{14}CH_3$ in experiments on rats

ADLER, ELLIOTT & GEORGE (1957) used radioactive technique to study the fate of morphine in the rat Morphine was isolated by paper chromatography, after which it was converted to crystalline dinitrophenylmorphine-N-$^{14}CH_3$ before the radioactivity was measured The method is thus exceedingly specific, and the sensitivity was satisfactory, although it appears from the results presented that the uncertainty of the method is very considerable

MULÉ & WOODS (1962) and MULÉ, WOODS & MELLETT (1962) used morphine-N-$^{14}CH_3$ in experiments on dogs JOHANNESSON & WOODS (1964) with a few modifications, have used the same technique in experiments on rats The measurements were based on fluid scintillation technique The lowest measureable amounts were around 5 ng Increasing amounts of morphine (10–1500 ng) gave a linear rise in the intensity of the radioactivity measured ("counts per minute") On addition of morphine to homogenates of rat brains the recovery was about 93% ± 2% (limits of error, P 0 95)

A critical question in radioactive measurements on biological material is whether the radioactivity measured is localized in the molecule of the substance whose amount it is desired to determine Morphine-N-$^{14}CH_3$ can undergo N-demethylation in the organism (cf chapter IV A) If N-demethylation of morphine-N-$^{14}CH_3$ takes place to a great extent, there is no guarantee that the radioactivity measured corresponds to authentic morphine MULÉ & WOODS (1962) and MULÉ, WOODS & MELLETT (1962) examined whether normorphine or other metabolites were present in the brain following the

administered 5 mg/kg morphine-N-$^{14}CH_3$ by subcutaneous injection to 16 rats The rats were divided into 4 groups of 4 animals each The rats in these 4 groups were killed after 15, 30, 60 and 150 minutes, respectively Exactly the same procedure was used in experiments with radioactive codeine (25 mg/kg s c) and methadone (3 mg/kg s c) However, the methadone experiment was continued for 180 minutes after the injection The spinal cord and the brain were removed and determinations made of morphine, codeine or methadone in the spinal cord, hypothalamus, cerebellum, medulla oblongata mid brain and a part of the hemispheres The concentration of morphine rose strongly from the 15th to the 30th minute, while a weaker rise was found from the 30th to the 60th minute The greatest concentration was thus measured 60 minutes after administration 150 minutes after the injection the concentration of morphine was lower than after 30 minutes, except in the mid brain and in the cerebellum The course of the codeine amounts with time was in general the same as with morphine However, the concentration of codeine was relatively much lower after 150 minutes than was the case with morphine The concentration of methadone in the brain was found to be greatest 30 minutes after administration For comparison, it might be mentioned that JÓHANNESSON & SCHOU (1963 a) administered 20 mg/kg morphine to rats by intraperitoneal injection, and measured the concentration in the brain 15, 30, 45 and 60 minutes after the administration The results showed that the concentration of morphine was greatest 30 minutes after the injection Summarizing, therefore, it may be concluded that the concentration of morphine and codeine in the rat brain and the analgesic effect of these substances appear to culminate at approximately the same time (cf chapter I C)

The distribution of codeine was very uniform in all the parts of the brain and spinal cord studied in the experiments by MILLER & ELLIOTT Methadone was also distributed quite uniformly in all the sections examined Morphine however was found in considerably greater concentration in the spinal cord and in the hypothalamus than in the other parts of the central nervous system The results can thus suggest that morphine is not distributed quite so uniformly in the central nervous system as codeine and methadone In this connection it is of considerable interest to note that MULÉ & WOODS (1962) investigated the distribution of morphine in the brain of the dog after injection of 2 mg/kg morphine-N-$^{14}CH_3$ They found that within the first 4 hours the concentration was considerably greater in the grey matter than in the white matter Morphine was apparently very uniformly distributed in the grey matter and white matter, respectively These authors also found that the spinal cord and the hypothalamus showed no greater concentration of morphine than the other structures

The analgesic effect (the method of D'AMOUR & SMITH see page 21) of

(1950) and LATHAM & ELLIOTT (1951) used codeine-O-$^{14}CH_3$ *to investigate* the metabolism of codeine in the rat. The determinations were semi quantitative.

MILLER & ELLIOTT (1955) used codeine-N-$^{14}CH_3$ in experiments on the rat. The results were expressed quantitatively. These authors did not state the recovery percentage in the various tissues or the least amounts which could be determined quantitatively. According to WAY & ADLER (1962), the sensitivity and specificity of the method are high.

JOHANNESSON & WOODS (1964) used codeine-N-$^{14}CH_3$ (prepared by a modification of the method of ANDERSEN & WOODS 1959) in experiments on rats. Amounts down to about 5 ng could be determined quantitatively. Codeine was added to brain homogenates and to plasma in various amounts (100–6000 ng). A recovery of about 96 ± 2 % (limits of error, $P = 0.95$) was obtained.

Codeine can undergo O-demethylation to morphine in the organism (cf. chapter IV B). JOHANNESSON & WOODS (1964) therefore attempted to distinguish between codeine, and morphine formed from codeine in the organism. In experiments *in vitro*, it was found that morphine could be separated quantitatively from codeine, if the amount of morphine constituted more than 3–4 % of the amount of codeine present. The uncertainty in the determination of morphine in the presence of codeine was considerably greater than when morphine was determined alone. The method is thus subject to the error that small amounts of morphine-N-$^{14}CH_3$ which might be present in the brain after the administration of codeine-N-$^{14}CH_3$ cannot be distinguished from codeine.

Codeine-N-$^{14}CH_3$ can be converted to norcodeine in the organism (ADLER 1952). If this happens, part of the radioactivity measured might well be localized at other sites than at the aminomethyl group of the codeine molecules. However, there does not appear to be any proof that norcodeine is present in the brain of rats following administration of analgesic doses of codeine (cf. chapter IV C). It might be mentioned here that the ability of the rat brain to N-demethylate codeine is extremely slight *in vitro* (ELISON & ELLIOTT 1963).

B. Changes in the amounts of morphine and codeine in the brain with time, and their distribution in the brain

There appear to be only two investigations which are concerned with the changes in the amounts of morphine and codeine in the brain during the time elapsing after their administration to rats, and only in one study, namely the paper of MILLER & ELLIOTT from 1955, has an attempt been made to determine the distribution within the brain of the rat. These authors

administration With the radioactive technique used (see page 32), the morphine concentration in the brain, which could be measured in all the animals was on an average 183 ng/g tissue (130 – 225 ng/g) The experimental results thus suggest that 30 minutes after intraperitoneal administration of morphine, in doses which produce an analgesic reaction in 70–80% of the rats, the morphine concentration in the brain can lie between about 0 2 and about 0 6 μg/g.

The analgesic effect is greatest 30–60 minutes after the administration (cf chapter I C) The morphine concentration also seems to reach a maximum in this interval (cf the previous section, chapter II B) Thus during this interval, it should be possible to determine both the strength of the analgesic effect and the concentration of the substance in the brain with a greater certainty than at any other time Experiments aiming at a comparison of the analgesic effect of morphine and the concentration of the drug in the brain should preferably be carried out within this time interval In this connection it should be emphasized that JÓHANNESSON & WOODS (1964) found the same analgesic effect 30 minutes after subcutaneous administration of 5 mg/kg morphine as after 10 mg/kg administered intraperitoneally At this stage, the morphine concentration in the brain was found to be the same in the rats in both experimental groups (180 ng/g) These results thus suggest that under given experimental conditions, a close relationship is found between the degree of the analgesia and the concentration of morphine in the brain Further experiments are necessary, however, to provide more information on whether the degree of analgesia depends directly on the concentration of morphine in the brain as a whole (cf also page 65)

Thirty minutes after the administration of equianalgesic doses of codeine, given subcutaneously (60 mg/kg) and intraperitoneally (30 mg/kg), respectively, JÓHANNESSON & WOODS (1964) found 9000 and 7900 ng/g codeine in the brain These two mean values did not differ significantly The doses of codeine mentioned were equianalgesic with 5 mg/kg morphine administered subcutaneously and 10 mg/kg given intraperitoneally As mentioned, a mean value of 180 ng morphine per g brain was found 30 minutes after the administration of these doses

With the same analgesic effect, the concentration of codeine in the brain is thus 45–50 times greater than that of morphine Here, however, it must be emphasized that the question is not elucidated whether the analgesic effect of codeine is due to the unconverted molecule alone, or whether morphine which has been formed from codeine in vivo also plays a role in the effect (cf chapter IV B)

The above experimental results make it possible to arrive at an estimate of both the relative and the absolute amounts of morphine and codeine in the brain Reckoning with a brain weight of 1 7 g in the rat with a body weight

morphine, codeine and methadone was also measured in the experiments by MILLER & ELLIOTT The rats, however, reacted with "complete analgesia" as early as 30 minutes after the administration, and still did so after 120 minutes The analgesic effect was thus not a graduated one In the study by MILLER & ELLIOTT, therefore, there is no experimental basis for a comparison between the concentration of these substances in the brain and the degree of analgesia (cf next section)

C. Morphine and codeine in the brain after the administration of analgesic doses

Whatever the form of the pharmacologic test, it is highly desirable to be able to compare the effect of a given substance and its concentration in the effector organ The point at which the analgesic effect is exerted is not precisely known, although in the first instance, the action appears to be localized to certain structures in the brain (cf chapter I A) As mentioned above, fairly constant ratios will be found between the concentration of morphine and codeine in the various parts of the brain It would therefore appear permissible, in tests with a definite species of experimental animal, to compare the analgesic effect of these substances with their concentration in the brain as a whole Such investigations have been performed in rats by JOHANNESSON & SCHOU (1963 a, b) and later by JÓHANNESSON & WOODS (1964) The results of these studies will be described in further detail below

In the first study mentioned, attempts were made to determine the concentration of morphine in the brain of rats after intraperitoneal injection of respectively 5 0 and 8 5 mg/kg of the drug (JÓHANNESSON & SCHOU 1963 a) The analgesic effect was determined 30 minutes after the injection, and the rats were then killed immediately thereafter The amounts mentioned produced analgesia in 37 5% and 70% of the animals, respectively However in spite of examining two brains together as a rule, the results showed that the concentration of morphine in the brain of certain of the animals was below the level of sensitivity of the polarographic method used for quantitative measurements (see page 31) In both groups the measurable amounts lay between 0 20 and 0 54 μg/g brain, with a mean value of about 0 24 μg/g In other experiments, JÓHANNESSON & SCHOU (1963 b) found that 10 mg/kg morphine administered intraperitoneally produced analgesia in 80% of the rats 30 minutes after the injection The concentration of morphine was on the average 0 62 μg/g brain (0 50 – 0 74 μg/g) which was considerably more than in the previous experiment In both experiments, however, only relatively few measurements are available In tests with rats of another strain JÓHANNESSON & WOODS (1964) found that 10 mg/kg of morphine administered intraperitoneally produced analgesia in 70% of the rats 30 minutes after the

tions on the duration of tolerance and for studies on the concentration of the drug in the brain of tolerant animals following administration of morphine codeine and similar substances In what follows it will be tolerance to the *analgesic* effect of morphine which in general will be the topic for discussion, since tolerance to other effects falls outside the scope of the present study

A. Development of tolerance

JOËL & ETTINGER (1926) were the first to study how rapidly tolerance can develop to the analgesic effect of morphine in rats They pinched animals in the tail and studied the reaction to the painful stimulus The first reaction was a squeak After administration of 100 mg/kg (s c) the rats could be pinched without reacting in this way The effect lasted more than 8½ hours in control animals but it was of considerable shorter duration in rats which had previously received morphine daily for some time The difference was noticeable as early as after 3–4 days The technique of JOËL & ETTINGER was naturally very primitive but their results nevertheless clearly demonstrate that the development of tolerance can appear after a few doses, and show itself by shorter duration of the morphine effect

The development of tolerance to the analgesic effect in rats was also studied by LEWIS (1949), using the method of ERCOLI & LEWIS (1945) for measuring analgesia (see page 21) In tests with analgesics a prolongation of the reaction time of the animals (i e the reaction time without the administration of analgesics) by more than 100% was designated as "complete analgesia LEWIS performed experiments with 20 rats, which received 10 mg/kg morphine administered by subcutaneous injection daily for 6 weeks At first this dose produced "complete analgesia" in the rats Three weeks later this dose was found to prolong the reaction time of the rats by about 65%, while at the termination of the experimental period it was only able to prolong the reaction time by about 35%. In a sim[illegible] ment, WINTER [illegible] taneous injec- [illegible] the effect of [illegible] decreased although there was still a slight analgesic effect PÓRSZÁSZ, TARDOS, HERR & NYRI (1953) administered 8 mg/kg morphine daily by subcutaneous injection At first 75% of the rats gave an analgesic reaction to this dose, while only 50% of the animals reacted 4 weeks later After a period of 5 weeks however, at the end of the experimental period 8 mg/kg morphine was quite without analgesic effect HERKEN NEUBERT & TIMMLER (1959) administered daily 10 mg/kg morphine by subcutaneous injection for a period of 4 weeks The analgesic effect of this dose fell strongly during the first 2 weeks while the effect remained almost unchanged during the second half of the experimental period On the

of 200 g the amount of morphine found in the brain of a rat 30 *minutes* after morphine administration (5 mg/kg s c and 10 mg/kg i p) will be respectively, 0 03 % and 0 015% of the amount of morphine administered Corresponding figures for the equianalgesic doses of codeine (60 mg/kg s c, and 30 mg/kg i p) are, respectively, 0 13% and 0 23% These figures thus show that codeine enters the brain much more easily than morphine Summarizing it may nevertheless be concluded that only a relatively very small proportion of the morphine and codeine administered enters the brain It is undoubtedly *these extremely small amounts, however, which are decisive for* the analgesic effect of these drugs The results hitherto also suggest that there is a relationship between the concentration in the brain and the degree of analgesia

III. Tolerance to the analgesic effect

By *tolerance* to morphine or a morphine like substance is understood a *habituation (i e decreased susceptibility) to one or more of the* pharmacologic effects of the substance, acquired as a result of administration of the substance in question over a shorter or longer period of time By *abstinence symptoms* is understood those symptoms which can arise by sudden withdrawal of morphine or a morphine-like substance, following its administration for a shorter or longer period of time

An early and clear presentation of the concept of morphine tolerance in rats was given by RUBSAMEN (1908) He administered morphine in increasing amounts, or *until the animals were "morphineimmune" RUBSAMEN thus* wrote "Ich bezeichne eine Ratte als morphinimmun, wenn sie nach Immunisierung mehr als die von mir als absolut letal ermittelte Dosis von 60 mg pro 100 g Ratte erträgt, ohne zu sterben, ja ohne bemerkenswerte Erscheinungen der akuten Morphinvergiftung zu zeigen" In the almost 60 years which have since elapsed, tolerance to morphine in rats has in accordance with RUBSAMEN's procedure generally been produced by administration of the drug in large doses, often for weeks An example of this from the somewhat *older literature is HILDEBRANDT's paper from 1922, and* as examples from the last decade can be mentioned AXELROD (1956 b), SZERB & MC CURDY (1956), COCHIN & AXELROD (1959), GUNNE (1959), JÓHANNESSON (1962 a, b), MAYNERT & KLINGMAN *(1962)*, MARTIN *et al (1963)* and UNGAR (1965) Rats *can* therefore undoubtedly acquire a very pronounced tolerance to the analgesic, sedative, respiratory-depressant, hypnotic, lethal and possibly other effects of morphine It was not until the last few years however, that systematic studies were performed in order to determine the *least amounts* of morphine and the *shortest time* required to develop tolerance to certain of the effects of the drug The same holds for investiga-

morphine injections was identical Thus, while tolerance did not develop following the single injection of this smaller (analgesic) dose of morphine, as was the case in the experiment by COCHIN & KORNETSKY following administration of a single large dose, there can be no doubt that tolerance devel ops after a few repeated injections of analgesic doses

In the same investigation JOHANNESSON *et al* (1965 b) also examined the analgesic reaction expressed by the average reaction time in seconds (cf page 25) for rats treated as mentioned above A corresponding experiment was performed with codeine in equianalgesic doses (60 mg/kg s c) The analgesic effect was determined 30, 60 and 90 minutes after each injection in the morphine experiment, and 30, 60, 90 and 120 minutes after each injection in the codeine experiment Compared with the reaction time of a control group (water alone given), the analgesic effect at all the times mentioned was found to be less after the second than after the first injection Thirty and 60 minutes after the second dose of morphine, and 30, 60 and 90 minutes after the second dose of codeine, the reaction time was nevertheless still significantly different ($P < 0\ 05$) from the reaction time in the control animals, while with one exception, this was not the case after administration of the third, fourth or fifth dose ($P > 0\ 05$) These results are in agreement with the earlier findings by ERCOLI & LEWIS (1945), as described above These experiments also show clearly that the development of tolerance can be recognized at an earlier stage if reaction time is used as an expression of analgesia than if a record is simply made of the number of animals showing an analgesic reaction according to the criteria employed

JOHANNESSON *et al* (1965 b) also examined the question whether the route of injection could influence the development of tolerance Morphine was therefore administered by intraperitoneal injection once a week for a period of 3 weeks (a total of 4 injections) in an amount (10 mg/kg) which has the same analgesic effect as 5 mg/kg administered subcutaneously The experiment gave almost the same result as the experiment described above, in which morphine was administered subcutaneously In another experiment 10 mg/kg morphine was administered intraperitoneally on 4 subsequent days The effect of the 2nd and 3rd doses was of a considerably shorter duration than the effect of the corresponding doses in the experiment where injections were given weekly The time interval between the injections can thus influence the development of tolerance

It is seen quite clearly from the results of the investigations by COCHIN & KORNETSKY and JOHANNESSON, ROGERS, FOUTS & WOODS, that a considerable tolerance can arise to the analgesic effect of morphine and codeine even after the administration of few and small doses It is therefore by no means certain that biochemical changes which can be demonstrated after prolonged administration of these substances (cf chapter IV and chapter VI) have any

termination of the experimental period, this dose thus had some analgesic effect although considerably slighter than at the start

The four studies mentioned above suggest that tolerance to the analgesic effect arises within a few weeks These studies are nevertheless unable to point to a more precise time of onset for the appearance of tolerance However, ERCOLI & LEWIS (1945) already drew attention to the fact that morphine and morphine-like substances did not have the same analgesic effect in rats on the second injection as on the first injection They thus wrote "It had been observed before that after an animal had once been treated with opiates its manner of reaction in future tests might be influenced by the previous treatment" According to the method of ERCOLI & LEWIS (see page 21), a prolongation of the reaction time in seconds after administration of analgesics is a criterion of the analgesic effect Their statement therefore suggests that the analgesic effect was shorter after the second than after the first injection, as JOHANNESSON *et al* (1965 b) found later (see below) However, they added no further comments to this GREEN *et al* (1951) likewise state that rats are to some extent tolerant to the analgesic effect following a single preceding administration of morphine or methadone

Within the last two years, however, two major studies have appeared in which an attempt has been made to determine more precisely the time of onset of tolerance to the analgesic effect, as well as the duration of the tolerance The first of these studies is that by COCHIN & KORNETSKY from 1964 These authors used EDDY's hot plate for determining analgesia in rats and expressed the analgesic effect in "minute-seconds" as described by WINTER & FLATAKER (1950, see page 21) They investigated the analgesic effect of morphine with time after a single large dose (20 mg/kg i p), and found that on renewed injection of the test dose after 88 days the intensity of the analgesia was about 40% of the initial intensity in "minute-seconds" The study by COCHIN & KORNETSKY is particularly concerned with the question of the duration of tolerance so that it will be mentioned further below (see page 43) The other study is by JOHANNESSON, ROGERS, FOUTS & WOODS (1965 b), in which these authors investigated the development of tolerance after repeated small (analgesic) doses of morphine Five injections of 5 mg/kg were administered subcutaneously at intervals of a week, and the analgesic effect measured 30 minutes after each injection On the first occasion 75% of the animals reacted with analgesia according to the criteria employed The analgesic reaction, however, became gradually reduced from week to week, although the reduction was not statistically significant until after the fifth injection ($P < 0.05$) A single group of animals received injections and were tested in the same way as the other animals but they received morphine only on the first and last injection while the other three injections consisted of water alone The analgesic reaction following the two

morphine injections was identical. Thus, while tolerance did not develop following the single injection of this smaller (analgesic) dose of morphine, as was the case in the experiment by COCHIN & KORNETSKY following administration of a single large dose, there can be no doubt that tolerance develops after a few repeated injections of analgesic doses.

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It is seen quite clearly from the results of the investigations by COCHIN & KORNETSKY and JÓHANNESSON, ROGERS FOUTS & WOODS, that a considerable tolerance can arise to the analgesic effect of morphine and codeine even after the administration of few and small doses. It is therefore by no means certain that biochemical changes which can be demonstrated after prolonged administration of these substances (cf. chapter IV and chapter VI) have any

significance for the development of tolerance to the analgesic effect These biochemical changes should rather be related to the abstinence symptoms which develop in rats after the discontinuation of chronic administration of morphine or codeine MARTIN *et al* (1963) have provided a detailed description of the abstinence symptoms which develop in rats following the termination of chronic administration of morphine in large doses There has been apparently no investigation of those minimal doses which can be followed by development of abstinence symptoms in rats WIKLER & CARTER (1953) in experiments on reflexes in spinal dogs (spinal cord transected at the level of C_7–C_8), found that those changes which are taken as expressing abstinence symptoms in these animals can be demonstrated already after a few injections of morphine It is possible, therefore, that abstinence symptoms in one form or another might be recognized after the administration of a few analgesic doses of morphine or codeine Abstinence symptoms of the type which can be recognized and recorded following the administration of the least possible doses of morphine or codeine have thus the greatest chance of possessing a common mechanism with the development of tolerance to the analgesic effect

Whether tolerance to other effects of morphine develops just as early as tolerance to the analgesic effect is a question which has not yet been elucidated It should be emphasized in this connection that KOKKA, ELLIOTT & WAY (1965), after the intraperitoneal administration of 10 mg/kg morphine to rats during 4 consecutive days were able to demonstrate a considerable tolerance to the respiratory-depressant effect LOTTI, LOMAX & GEORGE (1966) have also recently found that considerable tolerance to the hypothermic effect of morphine can be demonstrated in rats in the course of a few hours after a single administration of large doses (15–20 mg/kg i v) With respect to the lethal effect of morphine, tolerance to this effect is not found following the administration of morphine in a few analgesic doses (JÓHANNESSON *et al* 1965 b), although it is known that such tolerance develops after prolonged administration (JÓHANNESSON 1962 a, b)

B. Duration of tolerance

It is difficult to investigate the duration of tolerance, since each single administration of morphine or morphine-like substances necessarily contributes to the maintenance of the tolerance

In humans, tolerance to the analgesic and lethal effects of morphine appears to be of short duration (cf EDDY 1955) In monkeys (macacus rhesus) tolerance to the lethal and the hypnotic effects of morphine codeine and heroin disappears in the course of 2 weeks after termination of the administration (KOLB & DU MEZ 1931) LEWIS (1949), in experiments with rats, found

similar results Three weeks after daily administration was discontinued, morphine had about the same analgesic effect as when it was first administered This is in contrast to the findings of other authors JOEL & ETTINGER (1926) thus found that tolerance to morphine lasted for at least 30 days ("Noch nach 30 Tagen Giftkarenz unterscheiden sich ehemals gewohnte Tiere deutlich von unvorbehandelten") – The experimental conditions, however, are hardly comparable

By far the most thorough investigation of the duration of morphine tolerance has been made by COCHIN & KORNETSKY (1964), and their results will therefore be discussd further As already mentioned, they found that even three months after a single intraperitoneal administration of 20 mg/kg morphine, considerable tolerance to the analgesic effect still prevailed The experiments comprised rats in 6 groups The rats in group 1 received a test dose (20 mg/kg i p) administered 3, 4 6, 8, 11 and 15 months after the first injection, and the analgesic effect was determined after each injection Rats in groups 2–6 were again taken into experiment at 4, 6, 8, 11 and 15 months, respectively, after the first injection It was thus possible to follow *the time course* of the tolerance very closely The results showed that tolerance was still unchanged 11 months (group 5) after a *single administration* of morphine After fifteen months, however, the analgesic effect (group 6) was the same as after the first injection The tolerance must therefore have disappeared within the period between 11 and 15 months after the first injection In the rats of group 1, in which 20 mg/kg morphine was administered *several times* at intervals of months the tolerance if anything increased in strength COCHIN & KORNETSKY worked with control groups in order to exclude the possibility that the *time factor* or the procedure for determining analgesia (EDDY's hot plate) should influence the reactivity of the experimental animals and thereby the results The experiments were moreover in part performed in rats of two strains so that the results would presumably have a general validity for this species On the basis of these extremely thoroughly and carefully performed experiments the conclusion can be drawn that tolerance to the analgesic effect of morphine may have a particularly prolonged course in the rat It is still not clear whether tolerance to the analgesic effect of codeine and other *euphoric* analgesics is of as long a duration as tolerance to morphine In advance, however, this must be considered as likely

C. Morphine and codeine in the brain of tolerant rats

Only a few studies have been made which are concerned with the question of the occurrence of *morphine* and codeine in the brain of tolerant rats The first study of any importance appears to have been made by SZERB & MC CURDY (1956), who administered 75 mg kg morphine intravenously to non-

tolerant and tolerant rats (previously treated with morphine in increasing amounts over a period of 4 weeks) Morphine was determined in the brain 10, 60 or 180 minutes after the administration Sixty minutes after the injection, the concentration of morphine was found to be lower in the tolerant rats than in the non-tolerant rats ($P < 0\ 01$) The results thus suggested that morphine disappears more rapidly from the brain in tolerant rats than in non-tolerant rats Similar relationships also appear to hold in the case of morphine tolerant dogs (MULÉ & WOODS 1962) Both SZERB & MCCURDY and MULÉ & WOODS, however, emphasized that the differences demonstrated did not explain tolerance to morphine in dogs and rats, respectively

JOHANNESSON (1962 a) administered large amounts of morphine to non-tolerant and tolerant rats (previously treated in almost the same way as in the experiments of SZERB & MCCURDY) Fourteen out of 19 non-tolerant rats died in the course of 40 minutes and only one rat survived 60 minutes Out of 16 tolerant rats, 3 died in the course of 40 minutes, while the others were killed respectively 35, 40 and 60 minutes after the administration The concentration in the brain was found to be between 20 and 40 µg/g in rats in both groups Tolerance to the lethal effect of morphine can therefore hardly be explained by lower concentration in the brain

JOHANNESSON & SCHOU (1963 b) and JOHANNESSON & WOODS (1964) studied the analgesic effect of morphine and codeine in tolerant and non-tolerant rats, and simultaneously determined the concentration of these substances in the brain JÓHANNESSON & SCHOU found that ED_{50} for morphine administered intraperitoneally was 7 mg/kg in non-tolerant rats, while in morphine tolerant animals it was about 20 mg/kg Following the administration of the same dose of morphine (10 mg/kg i p) to non-tolerant and tolerant rats, the concentration was found to be of the same order of magnitude in the two groups JÓHANNESSON & WOODS (1964) likewise administered 10 mg/kg morphine by intraperitoneal injection to non-tolerant and tolerant rats, and determined the analgesic effect after 30 minutes While 69% of the non-tolerant rats showed an analgesic reaction, only 11% of the morphine tolerant rats reacted with analgesia The morphine concentration in the brain of the rats, however was the same in both groups Following the administration of equally large doses of codeine, the mean concentration was found to be the same in the brain of non-tolerant and codeine tolerant rats Tolerance to the analgesic effect of morphine and codeine in the rat can thus not be explained by a lower concentration in the brain as a whole

There appear to be no studies of whether the distribution of morphine and codeine is the same in the brain of tolerant and non-tolerant rats MULÉ & WOODS (1962), however, found in their experiments on dogs that the distribution of morphine in the brain was more or less the same when given to morphine tolerant and non-tolerant animals As already mentioned, the

only notable difference in the fate of morphine in the brain of tolerant and non-tolerant dogs in these experiments was that the morphine disappeared more rapidly from the brain of the tolerant animals It should be emphasized in this connection that it is not known at all whether morphine and similar compounds are bound to the cells of the brain (and other organs) in the same manner, in tolerant and non tolerant animals Changes in the ability of the cells to bind these compounds can undoubtedly be of significance for the development of tolerance This assumption is compatible with the fact that in experiments with tolerant and non tolerant rats, the morphine concentration in the brain is found to be the same in animals in both groups, although the analgesic effect is far less in the tolerant animals than in the non tolerant animals (cf above)

IV. The significance of the metabolic products of morphine and codeine for the analgesic effect

A. N-demethylation of morphine to normorphine

Normorphine has been ascribed particular significance for the analgesic effect of morphine and for the development of tolerance Thus it has been maintained by BECKETT, CASY & HARPER (1956) that the analgesic effect is due to normorphine resulting from the N-demethylation of morphine *in vivo* In the same [illegible]
to morphi[illegible]
Both these [illegible] were ventilated before it was substantiated beyond doubt that normorphine was present following the administration of morphine so that essentially they are both based on theoretical considerations However, these hypotheses have aroused considerable attention, and still do so They have also given rise to many valuable investigations during the last decade In this study, the author therefore feels justified in discussing these hypotheses rather extensively In what follows, an account will first be given of the two hypotheses, and then they will be evaluated with respect to the experimental findings during the last few years

In an earlier study, BECKETT & CASY (1954) were interested in the space configuration of the hypothetical receptors in the central nervous system, presumed to be decisive for the analgesic effect BECKETT, CASY & HARPER (1956) then postulated that euphoric analgesics such as morphine, pethidine and methadone had to undergo N-demethylation on the receptor substance itself in order to exert their analgesic effect (cf also LOCKETT & DAVIS 1958) It is obviously difficult to prove or disprove such a hypothesis, as we are still on the whole ignorant of the mechanism of the analgesic effect How-

tolerant and tolerant rats (previously treated with morphine in increasing amounts over a period of 4 weeks) Morphine was determined in the brain 10, 60 or 180 minutes after the administration Sixty minutes after the injection, the concentration of morphine was found to be lower in the tolerant rats than in the non-tolerant rats ($P < 0.01$) The results thus suggested that morphine disappears more rapidly from the brain in tolerant rats than in non-tolerant rats Similar relationships also appear to hold in the case of morphine tolerant dogs (MULÉ & WOODS 1962) Both SZERB & MCCURDY and MULÉ & WOODS, however, emphasized that the differences demonstrated did not explain tolerance to morphine in dogs and rats, respectively

JOHANNESSON (1962 a) administered large amounts of morphine to non tolerant and tolerant rats (previously treated in almost the same way as in the experiments of SZERB & MCCURDY) Fourteen out of 19 non-tolerant rats died in the course of 40 minutes and only one rat survived 60 minutes Out of 16 tolerant rats, 3 died in the course of 40 minutes, while the others were killed respectively 35, 40 and 60 minutes after the administration The concentration in the brain was found to be between 20 and 40 µg/g in rats in both groups Tolerance to the lethal effect of morphine can therefore hardly be explained by lower concentration in the brain

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ever, the hypothesis assumes that normorphine cannot be a weaker analgesic than morphine

MISRA, MULÉ & WOODS (1961) and TANAKA (1961) were the first to demonstrate the presence of normorphine following the administration of morphine to rats Normorphine was isolated from the urine in both series of experiments The study by TANAKA (1961) is only available in the form of an extract, so that the further experimental details are not known to the present author (cf page 62) MISRA, MULÉ & WOODS (1961) performed their experiments with tritium-labelled morphine, and after subcutaneous administration of 10 mg/kg were able to demonstrate normorphine in the urine by paper chromatography A year later, MILTHERS (1962) reported that normorphine can be demonstrated in the brain of rats after the administration of morphine *MILTHERS gave morphine to normal and hepatectomized rats by intravenous administration Fifteen minutes after the administration of 100* mg/kg she found on the average 6 2 μg morphine and $<$ 0 4 μg normorphine per g brain in the control animals and 26 and 1 3 μg/g respectively, in the brain of hepatectomized rats Part of the extra-hepatic normorphine formation may have taken place in the brain (cf MILTHERS 1962) JOHANNESSON & MILTHERS (1963) also found that 15 and 30 minutes after the intraperitoneal administration of 500 or 600 mg/kg morphine to rats, there was a mean concentration of 21 μg morphine and 1 4 μg normorphine, respectively, per g brain tissue There is thus no doubt that normorphine can be formed *in vivo* following the administration of morphine to rats However, the results *show clearly that even after the administration of very large doses of* morphine, only small amounts of normorphine are found in the brain If normorphine is conceived as being of significance for the analgesic effect of morphine, it must be assumed that normorphine itself possesses a very strong analgesic effect

JOHANNESSON & SCHOU (1963 a) compared the analgesic effect of morphine and normorphine in rats 30 minutes after intraperitoneal administration The effect of morphine was 6 times stronger than that of normorphine The rats were killed immediately after determining the analgesic effect, and the morphine or normorphine was determined in the brain On the same level of analgesia, the brain concentration of normorphine was 3–6 times greater than that of morphine Normorphine must therefore have a considerably weaker effect than morphine Thus it is unlikely that normorphine plays any role in the analgesic effect of morphine Normorphine on the other hand, has been *found to be considerably more toxic than morphine* (cf JOHANNESSON & MILTHERS 1962) It is not known at all however whether normorphine which is formed from morphine *in vivo* can contribute to the lethal effect of morphine

The results in the experiments of JOHANNESSON & SCHOU (1963 a) thus

contradict the hypothesis of BECKETT, CASY & HARPER (1956) MILTHERS (1964) has raised the objection to the exper ments of JÓHANNESSON & SCHOU that morphine and normorphine were determined in total brain According to MILTHERS (1964), it is possible that morphine and normorphine are distributed differently throughout the brain The question as to a possible difference in the distribution of morphine and normorphine in the brain, however, is still quite obscure

It has been shown that normorphine can be N methylated to morphine *in vitro* (CLOUET RATNER & KURZMAN 1963) as well as *in vivo* (CLOUET 1963) CLOUET *et al* thus demonstrated the presence of N methyl transferase in the brain and the liver of rats which in the presence of a methyl-donor (methionine) can methylate normorphine to morphine CLOUET injected methionine-$^{14}CH_3$ into the cisterna magna of rats and normorphine 15 minutes later (1 36 mg/kg) The rats were killed after 25 minutes and the brains removed for analysis She found by paper chromatography that the radioactivity was collected at one point which had the same relative front as authentic morphine After the administration of methionine $^{14}CH_3$ alone, or methionine $^{14}CH_3$ plus pethidine, no radioactivity was found which had a relative front identical with morphine These experiments thus show clearly that normorphine can be converted to morphine in the organism in the presence of methyl-donor

It was found by AXELROD (1956 a) that *in vitro*, the microsomal enzymes in the liver cells in rats rabbits and guinea pigs can N-demethylate *morphine*, codeine, methadone and pethidine to the respective nor-compounds He found it probable that the enzymes in the microsomes of the liver cells had a similar configuration to that of the (hypothetical) analgesic receptors in the [illegible] nvesti- [illegible] dihy- [illegible] pethidine and cocaine He administered increasing amounts of morphine by intraperitoneal injection to rats over a period of 35 [illegible]

[illegible] group and the rats in the second group were killed 24 hours after the last daily administration The second half of the rats in the first group were killed 12 days after the morphine administration was discontinued The results showed that the chronic administration of morphine resulted in a considerable reduction *in* the ability of the liver microsomes to N-demethylate morphine, dihydromorphinone and pethidine The morphine administration had *no effect* on N-demethylation of cocaine The simultaneous administration of nalorphine reduced the morphine effect and twelve days after discontinuing the administration of morphine, the microsomal metabolism was the same as that in the control animals. The

results thus suggested that a reduction in the ability of the microsomes to N-demethylate euphoric analgesics could have a causal relationship with the development of tolerance to these substances It should be mentioned here that cross-tolerance exists between morphine, dihydromorphinone and pethidine The antagonistic effect of nalorphine and the absence of an effect on the cocaine metabolism suggested that the process was of a specific nature Furthermore, the reduced enzyme activity was apparently dependent on continued administration of morphine It therefore appeared to be a reasonable assumption that tolerance to morphine and morphine-like substances could be due to the absence or inactivity of receptors in the brain, since the microsomal enzymes and the hypothetical analgesic receptors were presupposed to have the same spatial configuration (cf AXELROD 1956 a) AXELROD (1956 b) formulated his hypothesis as follows "The continuous interaction of narcotic drugs with the demethylating enzymes inactivates the enzymes Likewise, the continous interaction of narcotic drugs with their receptors may inactivate the receptors Thus, a decreased response to the narcotic drugs may develop as a result of unavailability of receptor sites"

The original investigations by AXELROD were carried further by AXELROD & COCHIN (1957) and COCHIN & AXELROD (1959) COCHIN & AXELROD administered morphine in increasing amounts to rats over a period of 14 or 45 days The analgesic effect of 20 mg/kg morphine (i p) was determined at the start and at the end of the experimental period The results showed a pronounced parallelism between reduced N-demethylation in the hepatic microsomal enzymes, and the development of tolerance to the analgesic effect

AXELROD's conclusions were based on the hypothesis by BECKETT CASY & HARPER Quite apart from the fact that on the whole this hypothesis lacks an experimental basis, important objections can be directed to the studies by AXELROD Thus, HERKEN, NEUBERT & TIMMLER (1959) were unable to find any close relationship between reduced N-demethylation in the liver microsomes and the development of morphine tolerance in rats CLOUET & RATNER (1964) compared the development of morphine tolerance and the ability of the liver microsomes to N-demethylate pethidine in rats The same enzymes N-demethylate morphine and pethidine, but the activity is greater using pethidine as a substrate This compound was therefore chosen for study In agreement with HERKEN *et al*, CLOUET & RATNER found that the development of tolerance to the analgesic effect, and changes in the enzyme activity of the microsomes, varied independently of each other Furthermore, they found that chronic administration of morphine also reduced the microsomal metabolism of enhexymal (hexobarbital) They concluded, therefore, that reduced enzyme activity in the hepatic microsomes following chronic morphine administration, was probably an unspecific reaction

JOHANNESSON, ROGERS, FOUTS & WOODS (1965 a b) have made detailed studies as to the specificity of the changes taking place in the hepatic microsomal enzyme activity following chronic administration of morphine or codeine in large doses A study was made of the ability of the microsomes to metabolize codeine, enhexymal (hexobarbital), aniline, aminophenazone, benzpyrene zoxazolamine, chlorpromazine, *para* nitrobenzoic acid and neoprontosil The results showed that following chronic administration of morphine or codeine the microsomal metabolism of all the substrates examined was strongly reduced With one exception the difference between the tolerant rats and the control animals was statistically significant ($P < 0.05$) JOHANNESSON *et al* however, found no changes in the enzyme activity of the microsomes if morphine or codeine was administered in analgesic doses once a week for a period of 3 to 4 weeks It has been mentioned previously (cf chapter III A) that tolerance to the analgesic effect of morphine or codeine can develop following the administration of these substances in a few small doses Summarizing it is possible to conclude on the basis of these experiments that changes in the enzyme activity of the liver microsomes are of an unspecific nature, and without causal connection with tolerance to the analgesic effect of morphine and codeine This conclusion is quite in line with the results of a previous study by WOODS (1954) In experiments on morphine tolerant and non tolerant dogs and rats, WOODS thus found *no support for the* assumption that morphine tolerance could be explained by *altered* metabolism or distribution of morphine in the organism In this connection, however it should be emphasized that in rats, the development of tolerance to the hypnotic effect of certain barbituric acid derivatives is apparently directly dependent on increased metabolic activity in the hepatic microsomes (cf DENEAU & SEEVERS 1964) The increased metabolic enzyme activity which can be demonstrated in liver microsomes following administration of barbituric acid derivatives thus appears to a considerable extent to explain tolerance to these substances in rats (DENEAU & SEEVERS 1964) Similar relationships also appear to hold for tolerance to clopoxide (chlordiazepoxide) in rats (HOOGLAND *et al* 1966)

Thus even though AXELROD s hypothesis hardly longer enjoys any sure experimental basis there can be no doubt that his work has been most inciting to others Long before AXELROD, CLOETTA (1903) had suggested a connection between the development of tolerance and the receptor substance in the cells CLOETTA however, imagined that morphine tolerance was connected with an increase rather than with a loss in the receptor substance He wrote 'Also nicht von einem Rezeptorschwund ware hierbei zu sprechen, sondern eher von einer Vermehrung bei gleichzeitiger Abnahme der Reizempfindlichkeit der Zelle fur die toxophore Gruppe des Morphins" Future hypotheses to explain morphine tolerance will undoubtedly be based, in part at

least, on considerations similar to those put forward by AXELROD and CLOETTA Thus, the present author is of the opinion that the ability of the cells to bind morphine and similar substances can differ in tolerant and non-tolerant animals (cf page 45)

B. O-demethylation of codeine to morphine

WOLFF (1938) already ventilated the idea that morphine addicts could form morphine from codeine WOLFF had in fact, observed that morphine addicts could be maintained on codeine in large doses Ten years later, SANFILIPPO (1948) put forward the hypothesis that the analgesic and narcotic effect of codeine is due to the conversion of codeine to morphine WAY & ADLER (1962) have discussed SANFILIPPO's hypothesis in greater detail

The conclusions reached by WOLFF and SANFILIPPO were based on theoretical considerations alone, as at that time there was no proof that codeine could be converted to morphine *in vivo* or *in vitro* This has been demonstrated in subsequent studies ADLER & SHAW (1952) have thus found in incubation experiments that slices of rat liver can form morphine from codeine, and AXELROD (1955) showed that liver microsomes from rabbits, rats, guinea pigs and dogs can O demethylate codeine to morphine The ability of the brain cells to O-demethylate codeine *in vitro*, however, is extremely slight in the rat (ELISON & ELLIOTT 1963) After administration of codeine, morphine has also been identified in the urine in humans (ADLER, FUJIMOTO, WAY & BAKER 1955), monkeys (WOODS, MUEHLENBECK & MELLET 1956) and dogs (PÆRREGAARD 1958 a) JOHANNESSON & SCHOU (1963 a) could also demonstrate morphine in the brain 15, 30, 45 and 60 minutes after intraperitoneal administration of large doses of codeine (75, 150, 200 mg/kg) to rats It should be emphasized that in these experiments, the morphine was isolated by means of paper chromatography and determined by the polarographic technique (see page 31) and that the presence of codeine does not constitute a disturbing factor in the analyses There seems to be no doubt, therefore that morphine was present in the brain of these animals

Morphine is a considerably stronger analgesic than codeine (cf chapter I D and chapter II C) It has therefore been of some interest to attempt to evaluate quantitatively whether the analgesic effect of codeine can be due to the morphine formed *in vivo* In experiments on rats, JÓHANNESSON & SCHOU (1963 a) have thus attempted to determine the amounts of morphine in the brain after administration of codeine in analgesic doses, and to compare these with the concentration in the brain after the administration of morphine in equianalgesic doses The results showed that morphine could not be determined quantitatively in the brains of all the animals It should also

be emphasized that even the measureable amounts of morphine were close to the limit for quantitative determinations with the technique employed. The measureable amounts of morphine in the brain however, were of the same order of magnitude, whether analgesia was due to morphine administration or to codeine administration. The results therefore suggest that morphine can be found in approximately the same amounts in the brain following the administration of either morphine or codeine in equianalgesic doses.

The investigations of JOHANNESSON & SCHOU (1963 a) were carried further by JÓHANNESSON & WOODS (1964). Here, the experiments were continued using codeine-N-$^{14}CH_3$ in rats. The rats received either 30 mg/kg by intraperitoneal injection or 60 mg/kg by subcutaneous injection. These doses are equianalgesic. Morphine could not be determined in the brain 30 minutes after (see below), while in plasma mean values of 226 and 524 ng morphine per ml were found after subcutaneous and intraperitoneal injection, respectively. The plasma concentrations differed significantly ($P < 0.05$).

With the technique used by JOHANNESSON & WOODS (1964), it was thus impossible to determine morphine in the brain although it occurred in plasma. As already mentioned (see page 34, chapter II A), the radioactive technique has the fault that small amounts of morphine cannot be distinguished from large amounts of codeine. After the administration of morphine and codeine in equianalgesic doses, the amounts of codeine in the brain are found to be 45–50 times greater than the amounts of morphine (see page 37, chapter II C). It is possible, therefore, that the small amounts of morphine which might have been present in the brain after the administration of codeine were determined as codeine. JOHANNESSON & WOODS assumed that the ratio between the morphine concentration in the brain and in the plasma was the same, whether morphine or codeine had been administered. Calculation showed that with morphine concentrations in the plasma of 226 and 524 ng/ml, respectively, amounts of 45 and 105 ng morphine per g brain tissue could be expected. It will be quite impossible to determine these amounts in the presence of the large amounts of codeine which occurred in the brain in these experiments. It should be mentioned here that ADLER (1962), in a very brief communication, has reported experiments in which she administered 33 mg/kg codeine-N-$^{14}CH_3$ subcutaneously to rats, and found morphine in the plasma and in the brain. The concentration of "free" morphine in the plasma was between 200 and 800 ng/ml. The concentration of morphine in the brain was not reported, but was apparently very slight ("The brain concentrations of morphine, while representing a very small fraction of the total C^{14} content of the tissue, are, nonetheless, compatible with the hypothesis that in the rat morphinemimetic effects of codeine are paralleled by biotransformation to morphine".)

In order to cast further light on the possible significance of morphine in the

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2) O methylation of morphine to codeine

ELISON & ELLIOTT (1964) administered 5 mg/kg morphine-N-$^{14}CH_3$ by subcutaneous injection into 4 rats and examined their total 24 hour urine By paper chromatography, a hitherto unidentified metabolic product was found From its position on the paper, this substance could be codeine ELISON & ELLIOTT then collected urine from 6 rats which had been given morphine *in increasing amounts* for 30 days In this way, such large amounts of the unknown metabolite were collected that its infrared spectrum could be determined The results of infrared spectrophotometry completely supported the previous supposition as to the identity of the metabolite It must therefore be considered as proved that morphine can be converted to codeine *in vivo* in rats The results on the other hand, suggested that only very small amounts of morphine were converted to codeine The findings by ELISON & ELLIOTT, therefore, can hardly be regarded as anything more than a curiosity

3) Conjugation with glucuronic acid

As mentioned previously (see pages 28–29), conjugation with glucuronic acid must be regarded as a pronounced process of inactivation and detoxication WAY & ADLER (1962) and MILTHERS (1964) have reviewed the topic

4) Pseudomorphine (oxidimorphine)

It was previously considered that morphine is converted to pseudomorphine *in vivo* (MARMÉ 1883, GÉRARD *et al* 1905) More recent studies *do not confirm these results* (MISRA, MULÉ & WOODS 1961, MULÉ, WOODS & MELLETT 1962) It must be regarded as unlikely, therefore that any morphine whatever is converted to pseudomorphine *in vivo*

V. The influence of cholinergic substances on the analgesic effect

On the basis of their own experiments, many authors have come to the conclusion that cholinergic substances can increase the analgesic effect of morphine and similar substances It has even been claimed that analgesia has a cholinergic mechanism, but other authors have been unable to confirm this Final clarity cannot be said to have been reached on this subject, but attempts will be made to elucidate it here by a discussion of the most important studies for and against this point of view

analgesic effect of codeine, JÓHANNESSON (1965) investigated whether nalorphine antagonizes the analgesic effect of morphine and codeine in the same way The substances were administered simultaneously by subcutaneous or intraperitoneal injection and the analgesic effect determined 30 minutes later Nalorphine was a stronger antagonist to codeine than to morphine Increasing doses of nalorphine increasingly antagonized the analgesic effect of a given dose of codeine, while this was not the case with morphine It is possible that in these experiments the nalorphine had a biphasic effect on the analgesic effect of morphine (cf chapter VII) The experimental results thus strongly indicate that the analgesic effect of codeine is not due alone to the morphine formed from codeine *in vivo* The demonstration of small amounts of morphine in the brain following the administration of codeine thus in no way signifies that the analgesic effect of codeine is due exclusively to morphine formed *in vivo* On the other hand, morphine formed *in vivo* could be regarded as contributing to the analgesic effect of codeine, as it is known that morphine and codeine administered simultaneously enhance each other's analgesic effect (JOHANNESSON 1965, JOHANNESSON & WOODS 1965, cf chapter I E)

C. Other metabolic products

1) N-demethylation of codeine to norcodeine

Norcodeine has been identified in the urine in man after administration of codeine (ADLER 1952, ADLER, FUJIMOTO WAY & BAKER 1955) AXELROD (1956 a) found that liver microsomes from rats and several animal species could N-demethylate codeine to norcodeine The brain cells possess some ability, although very slight, to N-demethylate codeine *in vitro* (ELISON & ELLIOTT 1963) ADLER (1963) found $^{14}CO_2$ in the expired air following subcutaneous injection of codeine-N-$^{14}CH_3$ into mice There is thus hardly any doubt that codeine can undergo N-demethylation in the organism in humans, rats and mice There are no experimental studies, however, to elucidate whether norcodeine occurs in the brain in humans or animals after administration of codeine

It has not been possible to find reports on analgesic experiments with norcodeine administered to rats In mice, norcodeine has been found to be a considerably weaker analgesic than codeine (MILLER & ANDERSON 1954, RAUSCH *et al* 1959) Norcodeine, therefore, can hardly be ascribed any significance for the analgesic effect of codeine

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V. The influence of cholinergic substances on the analgesic effect

On the basis of their own experiments, many authors have come to the conclusion that cholinergic substances can *increase* the analgesic effect of morphine and similar substances It has even been claimed that analgesia has a cholinergic mechanism but other authors have been unable to confirm this Final clarity cannot be said to have been reached on this subject, but attempts will be made to elucidate it here by a discussion of the most important studies for and against this point of view

analgesic effect of codeine, JOHANNESSON (1965) investigated whether nalorphine antagonizes the analgesic effect of morphine and codeine in the same way The substances were administered simultaneously by subcutaneous or intraperitoneal injection, and the analgesic effect determined 30 minutes later Nalorphine was a stronger antagonist to codeine than to morphine Increasing doses of nalorphine increasingly antagonized the analgesic effect of a given dose of codeine, while this was not the case with morphine It is possible that in these experiments the nalorphine had a biphasic effect on the analgesic effect of morphine (cf chapter VII) The experimental results thus strongly indicate that the analgesic effect of codeine is not due alone to the morphine formed from codeine *in vivo* The demonstration of small amounts of morphine in the brain following the administration of codeine thus in no way signifies that the analgesic effect of codeine is due exclusively to morphine formed *in vivo* On the other hand, morphine formed *in vivo* could be regarded as contributing to the analgesic effect of codeine, as it is known that morphine and codeine administered simultaneously enhance each other's analgesic effect (JOHANNESSON 1965, JOHANNESSON & WOODS 1965, cf chapter I E)

C. Other metabolic products

1) N-demethylation of codeine to norcodeine

Norcodeine has been identified in the urine in man after administration of codeine (ADLER 1952, ADLER FUJIMOTO, WAY & BAKER 1955) AXELROD (1956 a) found that liver microsomes from rats and several animal species could N-demethylate codeine to norcodeine The brain cells possess some ability, although very slight, to N-demethylate codeine *in vitro* (ELISON & ELLIOTT 1963) ADLER (1963) found $^{14}CO_2$ in the expired air following subcutaneous injection of codeine-N-$^{14}CH_3$ into mice There is thus hardly any doubt that codeine can undergo N-demethylation in the organism in humans rats and mice There are no experimental studies, however, to elucidate whether norcodeine occurs in the brain in humans or animals after administration of codeine

It has not been possible to find reports on analgesic experiments with norcodeine administered to rats In mice norcodeine has been found to be a considerably weaker analgesic than codeine (MILLER & ANDERSON 1954, RAUSCH *et al* 1959) Norcodeine, therefore, can hardly be ascribed any significance for the analgesic effect of codeine

JOHANNESSON & LAUSEN 1961; JOHANNESSON 1962 a) Irreversible cholinesterase inhibitors of the phosphostigmine type form stable compounds with the enzyme substance, which can only be split in quite special circumstances Dilution of the tissue or the addition of substrate (acetylcholine) will thus in general not change the degree of inhibition On the other hand, in the case of reversible cholinesterase inhibitors (for example morphine and chlorpromazine), both dilution of the tissue and addition of substrate will dissociate the enzyme-inhibitor compound to a greater or lesser degree In experiments with reversible cholinesterase inhibitors, therefore, the degree of inhibition depends on the dilution and the concentration of the substrate

It has been pointed out by BERNHEIM & BERNHEIM (1936), KUHN & SURLES (1938), and later EADIE (1941), all from Duke University in North Carolina, that morphine is a reversible cholinesterase inhibitor However, in later studies on morphine and cholinesterases, the reversibility of the inhibition has usually not been taken into account This holds for the studies of both LAVIKAINEN & MATTILA (1959) and SCHAUMANN (1959) These authors administered large amounts of morphine to rats and mice, after which they removed the brain and determined the cholinesterase activity in the brain homogenates The tissue was diluted 100 times, without a correction being made for the dilution, and the concentration of substrate used was furthermore particularly high (10^{-2}–10^{-3}M) It is understandable, therefore, that in these experiments, no reduced cholinesterase activity was found in the brain after the administration of morphine

In more recent experiments to elucidate the possible inhibition of the brain cholinesterases by morphine, JÓHANNESSON (1962 a) has allowed for the reversibility of the cholinesterase inhibition After administering large doses of morphine (600–780 mg/kg i p) to rats the morphine concentration was determined in the brain, and a correction was then made for the neces- [illegible]

In the experiments mentioned above, JÓHANNESSON (1962 a) used acetylcholine in such a small concentration (6.3×10^{-4}M), that dissociation of the compound betweeen morphine and the enzyme substance was to a large extent avoided According to JENSEN HOLM (1961, 1966) it is probable that the "physiological concentration" of acetylcholine is between 10^{-5} and 10^{-7}M The concentration of substrate used *should* thus be approximately of the same order of magnitude as that found in the brain *in vivo* The results of JOHANNESSON's experiments therefore strongly suggest that morphine *can* inhibit the cholinesterase activity of the brain *in vivo* The amounts of morphine administered, however, were as mentioned above very great (in fact, acute

BERNHEIM & BERNHEIM as early as 1936, and KUHN & SURLES (1938) *two years later, demonstrated that* cholinesterases in brain homogenates from experimental animals (including rats) were inhibited by emetine, apomorphine, morphine, codeine and several other morphine-like substances. BERNHEIM & BERNHEIM found that morphine and apomorphine inhibited the cholinesterases in considerably lower concentrations than was the case with the other substances. They therefore came to the conclusion that morphine and apomorphine could probably inhibit the cholinesterase activity in the brain *in vivo*. KUHN & SURLES *found a correlation between the emetic effect* of the substances and their ability to inhibit the cholinesterase activity of the brain *in vitro*. SLAUGHTER & MUNSELL (1940), in experiments on cats demonstrated that neostigmine increased the analgesic effect of morphine. Furthermore, in these experiments atropine was found to antagonize the *analgesic effect of morphine and of morphine administered simultaneously* with neostigmine. On the basis of their own experiments and the other experiments with morphine and brain cholinesterases mentioned above, SLAUGHTER & MUNSELL therefore came to the conclusion that morphine acts by means of a cholinergic mechanism. On the basis of experiments on human subjects, rabbits, rats, guinea pigs and mice, many investigators have since maintained that not only neostigmine, but also physostigmine, pilocarpine and carbacholine can increase the analgesic effect of morphine and similar substances (FLODMARK & WRAMMER 1945, CHRISTENSEN & GROSS 1948, SLAUGHTER 1950, KOMLÓS, PÓRSZÁSZ & KNOLL 1950, KNOLL & KOMLÓS 1951, PÓRSZÁSZ, KNOLL & KOMLÓS 1951, FROMMEL 1951, SZERB 1957, SAXENA 1958, GORDONOFF 1959, SCHAUMANN 1959 among others). However, few have expressed such strong support for the cholinergic hypothesis as GORDONOFF (1963), who performed experiments in rabbits. He wrote: "I can say on the basis of our numerous experiments that analgesia is strengthened and prolonged by cholinergic substances, and also by cholinesterase inhibitors." Others, on the other hand, have been unable to demonstrate *any synergism* with respect to the analgesic effect, in experiments *in* human subjects (ANDREWS 1942), guinea pigs (DE JONGH 1954), and rats (HERKEN, MAIBAUER & MULLER 1957, JÓHANNESSON & SCHOU 1963 b).

The majority of the studies mentioned thus clearly support the view that cholinergic substances can increase the analgesic effect of euphoric analgesics. *Nevertheless*, very weighty criticism can be directed against the original hypothesis of SLAUGHTER & MUNSELL (1940). In this connection, it is important in the first place to attempt to clarify whether morphine (and codeine) can be considered as inhibiting the cholinesterase activity of the brain *in vivo*. In experiments with cholinesterases and cholinesterase inhibitors, it is necessary to distinguish between irreversible and reversible cholinesterase inhibitors (*cf*

rats, found that neostigmine does not increase the analgesic effect of morphine On the basis of his own investigations, therefore, the present author is of the opinion that neostigmine has no influence whatever on the analgesic effect of morphine in rats However, the categorical statement (see above) by GORDONOFF (1963) suggests that neostigmine and other cholinergic substances always increase the analgesic effect in experiments on rabbits It might be mentioned here that neostigmine increases the lethal effect of morphine both in experiments on rats as well as on rabbits and that the synergistic effect can probably be explained by a combined central and peripheral respiratory paralysis (cf JOHANNESSON 1962 b)

VI. Noradrenaline and serotonin in the brain and the analgesic effect

The brain of mammals contains small amounts of noradrenaline and serotonin (5-hydroxitryptamine, also called enteramine) The brain also contains dopamine, which is converted by hydroxylation to noradrenaline (cf MØLLER 1965) Adrenaline is also found in the brain In rats, there is on the average about 0 40 µg noradrenaline, 0 60 µg serotonin and 0 70 µg dopamine per g brain, while the concentration of adrenaline is extremely low (< 0 01 µg/g) (BROWNLEE & SPRIGGS 1965) These substances, however, are not uniformly distributed throughout the brain as they are localized mainly to the diencephalon mensencephalon and the basal ganglia There is an extensive literature on the question of whether changes in the content of noradrenaline and serotonin in the brain play a part in the occurrence of certain pathological states as well as in the mechanism of action of a number of drugs (review ROBSON & STACEY 1962)

Following the administration of reserpine, the content of noradrenaline and serotonin in the brain and other organs is found to be considerably reduced within a few hours (BRODIE OLIN, KUNTZMAN & SHORE 1957, BERTLER 1961, MAGUS, KRAUSE & RIEDEL 1964), while the administration of monoamine oxidase inhibitors such as *iproniazid*, for example, strongly reduces the conversion of these substances to less active compounds (cf ROBSON & STACEY 1962) Iproniazid and other monoamine oxidase inhibitors can thus counteract the fall in the content of noradrenaline and serotonin in the brain after the administration of reserpine Reserpine and iproniazid have

lethal doses) The morphine concentration in the brain was therefore also very great, or on the average about 100 times greater than the concentration found in the brain following the administration of analgesic doses (cf chapter *II C)* *It is thus not very likely* that the analgesic effect of morphine can be due to a reduced cholinesterase activity in the brain In this connection it may be mentioned that a number of authors have been unable to demonstrate *any effect of irreversible* cholinesterase inhibitors of the phosphostigmine type on the analgesic effect (PORSZÁSZ, KNOLL & KOMLÓS 1951, SZERB 1957, SAXENA 1958, SCHAUMANN 1959) KNOLL & KOMLOS (1951) and SAXENA *(1958)* have furthermore been unable to confirm the demonstration by SLAUGHTER & MUNSELL (1940) that atropine antagonizes the analgesic effect of morphine It is also of interest in this connection that even prolonged administration of morphine neither affects the ability of the brain to split acetylcholine (JOHANNESSON 1962 a), nor does it influence the concentration of acetylcholine in the brain (JOHANNESSON & LONG 1964) The conclusion, *therefore*, must be *that it is highly improbable* that the analgesic effect has a cholinergic mechanism If it is the case that neostigmine physostigmine, pilocarpine and carbacholine increase the analgesic effect, this must be due to other factors

To elucidate this question, mention might be made of the experiments of KNOLL, KOMLOS & TARDOS (1953) and KOMLÓS & KOMLOS SZÁSZ (1954 a, b) *These investigators found that neostigmine in vitro* reduces both the ability of the serum proteins and of the liver (perfusion experiments on rat liver) to bind and inactivate morphine These authors therefore considered *that neostigmine and other cholinergic substances increase the concentration* of morphine in the brain by counteracting both the binding of morphine to the serum proteins as well as glucuronide conjugation in the liver Neither *KNOLL et al nor KOMLÓS & KOMLOS-SZÁSZ, however, performed experiments in vivo* SZERB & MCCURDY (1956), JÓHANNESSON (1962 b) and JOHANNESSON & *SCHOU (1963 b), on the other hand have determined the concentration* of morphine in the brain and blood of rats following simultaneous administration of morphine and neostigmine as well as after the administration of morphine alone The results of these three studies show unanimously that neostigmine does *not* increase the concentration of morphine in the brain or in the blood It should be emphasized that in the brain of rats, morphine is found exclusively as "free" morphine whether given alone or together with neostigmine (JÓHANNESSON 1962 b) It is thus overwhelmingly probable that KNOLL KOMLÓS & TARDOS (1953) and KOMLÓS & KOMLOS-SZÁSZ (1954 a, b) are incorrect in their assumption at any rate as far as concerns rats

As mentioned above, JOHANNESSON & SCHOU (1963 b), in experiments on

However, subsequent studies with morphine and reserpine have given exceedingly contradictory results TRIPOD & GROSS (1957), TARDOS & JOBBÁGYI (1958) and LEME & SILVA (1961), in experiments on mice, have thus found that reserpine increases and prolongs the analgesic effect, a result which DANDIYA & MENON (1963) have also arrived at in their experiments on mice and rats TAKAGI *et al* (1964) and MEDAKOVIĆ & BANIĆ (1964), in their experiments, found on the contrary that reserpine reduced the analgesic effect of morphine in mice and rats A number of authors have found it quite impossible to demonstrate any difference in the analgesic effect of morphine and codeine in reserpine treated and normal rats (TARDOS & JOBBÁGYI 1958, JOHANNESSON & SCHOU 1963 b, JÓHANNESSON & WOODS 1964) If the amount of noradrenaline and serotonin in the brain in mice is reduced by pretreatment with α methyl-3,4-dihydroxyphenylalanine (α methyldopa) or α-methyl 3-hydroxyphenylalanine (α methyl-*meta* tyrosine) (cf HESS *et al* 1961), the analgesic effect of morphine does not appear to be influenced (RUDZIK & MENNEAR 1965) In these experiments, only reserpine antagonized the analgesic effect. Similar findings have also been described in experiments with rats (MEDAKOVIĆ & BANIĆ 1964) It thus appears possible that reserpine in itself can influence the intensity of the analgesia and that this effect is not due to the release of noradrenaline or of serotonin in the brain This does not appear to support the hypothesis of SCHAUMANN Furthermore, it must be emphasized that only large doses (more than 20 mg/kg i p) or prolonged administration of morphine changes the content of adrenaline and other catecholamines in the brain (GUNNE 1959, SLOAN *et al* 1962, MAYNERT & KLINGMAN 1962), while the concentration of serotonin in the brain is not influenced at all by morphine (MAYNERT *et al* 1962, SLOAN *et al* 1963) In brief, therefore, it may be said that changes in the content of noradrenaline and serotonin in the brain can hardly explain the analgesic effect of morphine and similar substances If reserpine has an influence on the analgesic effect this is probably due to a direct action of the substance itself In this connection it might be mentioned that the sedative effect of reserpine in experiments on rats is apparently independent of the concentration of serotonin in the brain (MAGUS *et al* 1964)

It has already been mentioned that after prolonged administration of morphine changes take place in the content of catecholamines in the brain (noradrenaline, dopamine, adrenaline) Although somewhat outside the scope of the present section, brief mention will be made of some studies on the content of these substances in the brain, in tolerance and abstinence In this connection the investigation by SLOAN *et al* (1963) should be emphasized They administered increasing amounts of morphine to rats over a period of 40 days Two hours after the administration of the last dose, the content of catecholamines in the brain was found to be significantly greater than in the

been much employed as "tools" in investigating whether changes in the content of these physiological substances in the brain play a role in the mechanism of action of drugs In this connection it must be emphasized that reserpine and monoamine oxidase inhibitors, as mentioned affect both the amount of noradrenaline and the amount of serotonin in the brain It can therefore be difficult to decide whether the effect of reserpine and iproniazid should be related to changes in the content of one or both of these two physiologically active substances in the brain

It has been suggested that changes in the amount of noradrenaline and possibly also serotonin in the brain play a decisive role for the intensity of the analgesic effect of morphine and similar substances This hypothesis is based to a considerable degree on experiments carried out on animals which have been treated with reserpine These experiments will be discussed in greater detail

The first to perform experiments with morphine in animals treated with reserpine was SCHNEIDER (1954) He gave 10 mg/kg reserpine subcutaneously to mice 2 hours before the administration of morphine The results showed that reserpine reduced the analgesic effect considerably RADOUCO-THOMAS *et al* (1957), in experiments on guinea pigs, found that pretreatment with reserpine likewise reduced the analgesic effect of pethidine They concluded that the anti analgesic effect of reserpine was due to the effect of the substance on adrenergic substances and serotonin in the brain SCHAUMANN (1958) carried this work further As did SCHNEIDER (1954), he found that the administration of reserpine reduced the analgesic effect of morphine in mice He also found that pretreatment with iproniazid could weaken or abolish the reserpine effect on the analgesia In line with this DEFALQUE (1965) has recently reported that iproniazid can increase (potentiate) the analgesic effect of morphine in experiments on rabbits The results of the experiments of SCHAUMANN (1958) therefore suggested that the degree of the analgesia depends on the amount of noradrenaline and serotonin in the brain SCHAUMANN himself considered however that the antagonistic effect of reserpine was only associated with the release of noradrenaline He based this on the investigation of VOGT (1954) in which it was demonstrated that large doses of morphine could reduce the amount of noradrenaline and adrenaline in the brain of cats while no support was found in the literature for the claim that morphine reduced the amount of serotonin in the brain (see below) SCHAUMANN thus concluded "Morphine analgesia is mediated by a central liberation of noradrenaline which is abolished when the brain has been depleted of noradrenaline by reserpine" Support for the assumption of SCHAUMANN (1958) is provided by the observation that adrenaline and other sympathomimetic amines have been found to have an analgesic effect (IVY *et al* 1944, KAMEYAMA 1961)

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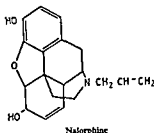
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control animals After 24 hours, the content of catecholamines in the brain was again normal In other studies, it has been found that morphine tolerant rats show excitation 2 hours after the last dose in the tolerance period, but this state subsequently changes to a phase of exhaustion, which is pronounced after 20 hours (JÓHANNESSON & LONG 1964, cf also MARTIN *et al* 1963) The changes in the concentration of catecholamines in the brain are thus in rather close agreement with these changes in the behaviour of the rats during the early period of abstinence It might be mentioned that during the period of abstinence, there are apparently no changes in the content of serotonin (SLOAN *et al* 1963) and acetylcholine (JOHANNESSON & LONG 1964) in the brain

VII. Nalorphine

POHL (1915) was the first to report the antagonistic action of N-allyl derivatives to morphine and morphine-like substances He performed experiments with morphine and N-allyl norcodeine in rabbits, and found that the norcodeine derivative in question was able to completely abolish the respiratory-depressant effect of morphine POHL furthermore emphasized that N-allyl norcodeine is a specific antidote to morphine, as he wrote "So macht es wenn ich das bisherige Material berucksichtige, fast den Eindruck, als ob das N-allylnorcodein ein nach Art eines specifisch eingestellten Antitoxins nur auf das Morphin wirke" However, POHL's experiments attracted only slight attention It was not until 25 years later that interest was aroused for the pharmacologic significance of substances possessing a powerful antagonistic action to the respiratory depression and certain other effects of morphine and similar substances

The first report on N-allyl normorphine, which was later given the name *nalorphine*, appeared in 1941 (HART 1941) Nalorphine is a considerably stronger morphine antagonist than N-allyl norcodeine It has been of great significance for the research on the mode of action of euphoric analgesics as well as in part in clinical work WOODS (1956) has reviewed the literature on nalorphine up to 1956 Later, WINTER, ORAHOVATS & LEHMAN (1957) in experiments on rats, showed that N-allyl and N propyl derivatives of morphine and morphine-like substances usually antagonize the analgesic effect of morphine In addition to nalorphine, naloxiphan (levallorphan) is the best known among these so-called anti-morphine substances



Nalorphine

Nalorphine itself possesses a number of morphine-like effects, but as a rule it is considerably weaker in its effect than morphine. In experimental animals, nalorphine has thus been found to have a weak (and to some extent uncertain) analgesic effect (WOODS 1956). This has later been confirmed (see, however, below) by among others WEISS & LATIES (1964) in experiments on monkeys, and HARRIS & PIERSON (1964) and JOHANNESSON (1965) in experiments on mice and rats, respectively. In man, on the other hand, nalorphine has a pronounced analgesic action (review: LASAGNA 1964). It is thus obvious that the results of analgesic experiments with animals in no way permit direct conclusions with respect to the conditions in man. In the most recent literature, however, there is a study by WINTER & FLATAKER (1965 b) which is of particular interest in this connection. These authors performed experiments in rats. They elicited pain (pain reaction: squeak) by mechanical stimulation of the hind paws of the rat. In the experiments, normal rats were employed as well as rats in which inflammation had been produced previously in the paws by local injection of 0.1 ml of a 5 % suspension of yeast. Without the administration of analgesics, the pain threshold was thus considerably lower in the animals in the latter group than in the animals in the former group. The results showed that nalorphine had no analgesic effect in the normal animals, while it had a pronounced analgesic effect in the pretreated animals, in which the pain threshold was pathologically reduced. It is possible, therefore, that the experimental technique of WINTER & FLATAKER opens new perspectives with regard to the evaluation of the analgesic effect of nalorphine and other anti-morphine substances in animal experiments (cf. pages 64–65).

In rats, nalorphine can be converted to normorphine *in vivo* (TANAKA 1961, MISRA, MULÉ & WOODS 1961, MILTHERS 1962, JOHANNESSON & MILTHERS 1963), and normorphine has been found to have a weak analgesic effect in experiments on these animals (JÓHANNESSON & SCHOU 1963 a). JOHANNESSON (1965) has therefore suggested that the weak analgesic effect of nalorphine in rats in these experiments may be due to its conversion to normorphine. In this connection, it should be emphasized that TANAKA (1961) has been able to demonstrate *morphine* in the urine of rats 2 hours after the

control animals After 24 hours, the content of catecholamines in the brain was again normal In other studies, it has been found that morphine tolerant rats show excitation 2 hours after the last dose in the tolerance period but this state subsequently changes to a phase of exhaustion which is pro nounced after 20 hours (JOHANNESSON & LONG 1964, cf also MARTIN *et al* 1963) The changes in the concentration of catecholamines in the brain are thus in rather close agreement with these changes in the behaviour of the rats during the early period of abstinence It might be mentioned that during the period of abstinence, there are apparently no changes in the content of sero tonin (SLOAN *et al* 1963) and acetylcholine (JOHANNESSON & LONG 1964) in the brain

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effect of nalorphine on morphine and codeine, respectively The author is therefore of the opinion that morphine formed *in vivo* does not play any great role for the analgesic effect of codeine (cf chapter IV B)

At the present stage, it is not possible to give any reasonable explanation for the contradictory results of experiments with morphine and nalorphine, described above It may be mentioned in this connection that the same method was used for determining analgesia (D'AMOUR & SMITH 1941) in three of the four studies mentioned, just as rats of the same strain (HOLTZMAN) were used in three of the four studies

Experiments on rats to determine the influence of nalorphine on the lethal effect of morphine and codeine, respectively, have likewise given contradictory results WINTER & FLATAKER (1956) thus found that nalorphine antagonizes the lethal effect of codeine, while JÓHANNESSON (1965) found that nalorphine rather increases than reduces the lethality KOPPANYI & KARCZMAR (1953) were unable to demonstrate any protection against the lethal effect of morphine following the administration of nalorphine JOHANNESSON & MILTHERS (1963) found furthermore that nalorphine was able to increase the lethal effect of morphine JOHANNESSON (1965) has later shown, however, that nalorphine can provide a high degree of protection against the lethal effect of morphine

In the above mentioned study, JOHANNESSON & MILTHERS found greater amounts of nalorphine in the brain after the administration of morphine plus nalorphine than after the administration of nalorphine alone JOHANNESSON (1963) took this question up to renewed investigation, since the experiments of JÓHANNESSON & MILTHERS (1963) served another purpose than to demonstrate possible differences in the nalorphine concentration in the brain In these experiments, JOHANNESSON administered both 320 mg/kg morphine alone, 150 mg/kg nalorphine alone, and finally these amounts of both substances by simultaneous, intraperitoneal injections into rats The animals were killed 30 minutes later The concentration of morphine in the brain was found to be an average of about 15 μg/g, no matter whether morphine was given alone or together with nalorphine Following the administration of nalorphine alone, the concentration of this substance in the brain was an average of 4 3 μg/g However, the nalorphine concentration was 13 0 μg/g when nalorphine was given together with morphine The difference was statistically significant It should be emphasized that the substances were separated by paper chromatography and determined by polarography

In agreement with JOHANNESSON's results, TANAKA (1961) found that a smaller amount of "free" nalorphine was excreted in the urine of rats after the administration of morphine with nalorphine, than after the administration of nalorphine alone As mentioned, the substances were apparently also separated by paper chromatography in these experiments In other experi-

administration of nalorphine TANAKA's original paper was published in Japanese, and only the English summary has been available to the present author It would appear from this that TANAKA has isolated morphine by means of paper chromatography, but it is difficult to form a clear idea of the experiments on the basis of this brief presentation

The available literature contains only few reports on rat experiments to elucidate the antagonism between nalorphine and the analgesic effect of morphine and similar substances The first investigations of this kind would appear to have been made by SMITH, LEHMAN & GILFILLAN (1951) They found that 10 mg/kg nalorphine given subcutaneously could abolish the analgesic effect of 6 mg/kg morphine and 2 mg/kg methadone, respectively All subsequent reports have confirmed that nalorphine antagonizes the analgesic effect of morphine to a greater or lesser degree COSTA & BONNYCASTLE (1955), however, constitute an exception In their experiments nalorphine had no effect on the analgesic action of morphine, while naloxiphan was found to have a weak antagonistic effect

It would be expected in advance that increasing doses of nalorphine would to an increasing degree antagonize the analgesic effect of a given dose of morphine WINTER *et al* (1954) and later HARRIS & PIERSON (1964) have in fact also found that the analgesic effect of morphine falls with increasing doses of nalorphine RUBIN, CHERNOV, MILLER & MANNERING (1964) and JÓHANNESSON (1965), however, have been unable to confirm these findings in their experiments RUBIN *et al* thus found that the antagonistic effect of nalorphine is greatest when the ratio between the amount of morphine administered (the dose was constant at 4 mg/kg i p) and the amount of nalorphine administered, is of a quite definite order of magnitude The results showed that the antagonistic effect was a maximum, when the ratio "dose of morphine/dose of nalorphine" was 1 3 On the other hand the analgesic effect was not reduced when the ratio was 1 0 and 4 0 Similar findings were also made in experiments with naloxiphan JÓHANNESSON (1965) investigated the antagonistic effect of nalorphine to the analgesic action of morphine and codeine Morphine or codeine were given in equianalgesic doses simultaneously with nalorphine, by subcutaneous or intraperitoneal injection Just as WINTER *et al* (1954) and HARRIS & PIERSON (1964) had previously found for morphine, the results of the codeine experiments showed that increasing amounts of nalorphine reduced the analgesic effect to an increasing degree The morphine experiments on the other hand, showed that there was no definite relationship between the amounts of nalorphine administered and the fall in the analgesic effect The results suggested however, that nalorphine has a biphasic action on the analgesic effect of morphine in line with the findings of RUBIN *et al* (1964) referred to above As mentioned there was a striking difference in JOHANNESSON's experiments between the

in animal experiments The experimental technique recently described by WINTER & FLATAKER (1965 b) is therefore of considerable interest in this connection (see page 61) As mentioned (page 61), nalorphine has a strong analgesic effect in man

It is only during the last decade that methods have appeared which can be characterized as both very sensitive and very specific, for the determination of morphine and codeine in biological material It is thus now possible, with a high degree of certainty, to determine the concentration in the brain of rats following the administration of analgesic doses However, when it is a question of determining morphine and codeine *in situ* in the brain or in other organs all the methods hitherto used, possibly with the exception of the very difficult autoradiographic technique, are found to be quite inadequate In this connection, fluorescence microscopy as well as electron microscopy should be considered The author is of the opinion that such methods, which should permit the demonstration of morphine in tissue sections, would among other things serve to elucidate whether the substance is deposited and bound in the same way in the tissues in tolerant as in non-tolerant animals

There is hardly any doubt that the analgesic effect of morphine and morphine like substances is localized to the central nervous system The locus at which the analgesic effect is exerted is not known precisely, although there are various facts which suggest that the action is localized to a high degree in the diencephalon One of the most important research topics for the future must thus be further elucidation of the question whether the analgesia is exerted at points which are mainly localized to certain definite parts of the brain

Morphine and codeine are more or less uniformly distributed in the brain It has therefore been considered justified to compare the degree of the analgesic effect and the concentration of the substances in the brain as a whole The results which have hitherto been obtained from such experiments suggest that here is a close relationship between the intensity of the analgesia and the concentration in the brain (page 37) Future experiments will help to elucidate whether the analgesia depends directly on the concentration, and in particular, on the concentration in certain parts of the brain, for example the thalamus

Previously tolerance to morphine was produced by the administration of the substance in increasing doses over a longer period of time More recent studies, however, show clearly that tolerance to the analgesic effect already exists after the administration of a few and small doses of the substance, while tolerance to the lethal effect is not recognized until larger doses have been administered It is thus hardly logical to proceed according to the same dosage system in studies of the development of tolerance to the analgesic effect as

ments, the present author has found that nalorphine can influence the concentration of morphine in the brain in rats (unpublished results) The same seems also to be the case in experiments with tolerant and non-tolerant dogs (MULÉ, WOODS & MELLETT 1962, MULÉ 1965) Further, these authors gave only small doses of morphine and nalorphine To summarize, therefore, it may be said that morphine and nalorphine can undoubtedly influence each other's distribution and excretion in experiments on dogs and rats Here, too, as so often before, it must be admitted that further experiments are necessary in order to elucidate the significance of these phenomena for the biological effects of these substances

VIII. General conclusions and future perspectives for research

The methods described in the literature for the determination of analgesia in animal experiments can be subdivided into af few groups depending on the kind of stimulation used to elicit pain Surprisingly many investigators, however have offered much time and trouble in modifying methods to determine analgesia It seems to be the rule rather than the exception that each individual investigator has used his own special modification of a method which was already known It is in the nature of things therefore, that it is often difficult to make direct comparisons of the results obtained by the various authors One must thus agree with WINTER (1965) when he writes "When one reads the literature on analgesic testing it sometimes seems that scarcely two workers use identical methods to elicit a response from the animals and to evaluate the data obtained"

The question which inevitably arises in this connection is whether the many modifications of the methodology can be said to be justified, and what aim they serve The question is no less actual as regards analgesic experiments on rats, since really comparative experiments have hitherto not been made with animals of this species It must nevertheless be regarded as necessary to perform such experiments, where the sensitivity and reproduceability of the different methods are studied under given experimental conditions in order to elucidate whether one definite method or modification of a method is preferable to other methods

The analges c effect of morphine or morphine-like substances can be demonstrated with certainty in animal experiments, but this is not usually the case with nalorphine and certain other so-called anti-morphine substances It is apparently necessary, therefore, to develop entirely new methods, if there is to be any hope of determining the analgesic effect of these substances

chanism of the analgesic effect and the phenomena of tolerance, have thus not only considerable theoretical interest but can also be of great practical significance in the battle against euphomania, which is a problem of considerable dimensions in many countries

Summary

SERTURNER's pioneering investigations of opium, leading to the isolation of morphine are mentioned in the introduction This is followed by an account of the extensive and often exceedingly contradictory literature on morphine and similar substances The concepts *analgesic effect* (analgesia) and *analgesic doses* are defined The author draws attention to the limitation imposed by the fact that his own experiments are performed exclusively in rats

Chapter 1

The analgesic effect of morphine and similar substances is localized to the central nervous system The exact site is not known, but the effect is apparently localized in particular to the diencephalon

Depending on the type of stimulation employed to elicit pain, methods for determining analgesia in animal experiments can be grouped into *mechanical electrical* and *thermal* methods The last group can be divided into two subgroups, based on stimulation by radiant heat and conducted heat, respectively The most important methods and modifications of methods are discussed in greater detail

No practical comparative analysis is available of the sensitivity and reliability of the different methods in experiments on rats It is therefore difficult to decide whether one method (or modification of method) is to be preferred to others However, the author considers that in choice of method, the thermal methods should receive particular consideration

Thirty to sixty minutes after subcutaneous or intraperitoneal injection the analgesic effect of morphine and codeine reaches a maximum The effect lasts up to *about 1½–3½ hours* after the administration The duration depends to some degree on the size of the dose Given subcutaneously, the effect of codeine lasts longer than that of morphine given subcutaneously, as well as that of morphine or codeine given by intraperitoneal injection Given intravenously, the substances have a powerful but very brief analgesic effect Given by mouth the substances have a slight effect

Morphine has a stronger analgesic effect given subcutaneously than when given intraperitoneally, while the reverse is the case with codeine, as this has a stronger effect after intraperitoneal injection than after subcutaneous injection When administered subcutaneously, morphine has thus *6–11 times*

to the lethal effect In future, it would appear reasonable to give morphine only in the minimal doses required to develop tolerance to just that effect which it is desired to investigate It should be emphasized at this point that we only have limited knowledge of the connection which presumably exists between the development of tolerance and the origin of abstinence symptoms As mentioned (see page 42), the form of abstinence symptoms *which can be demonstrated after the least possible doses of morphine have* the greatest possibility of possessing a common mechanism with the development of tolerance to the analgesic effect An important topic for future research must thus be to investigate and elucidate in greater detail just those abstinence symptoms which arise following the administration of the least possible doses of morphine This has already been emphasized by JOHANNESSON *et al* (1965 b)

Investigations of the metabolism of drugs are a relatively new branch of experimental pharmacology The microsomal metabolism of morphine and morphine-like substances has been the subject of numerous investigations during the last few years These investigations have already left deep impres*sions on studies on the mode of action of morphine and other euphoric anal*gesics, as the metabolic products have been ascribed a decisive significance for the analgesic effect and for the development of tolerance Although there is no definite support at present for the assumption that the metabolic products of morphine and codeine play an essential role for the action of these substances, the significance of the metabolic products in general must not be underestimated It is thus possible that the conversion of nalorphine to normorphine (or morphine) may be of significance for the action of this substance, both when administered alone and when administered together with morphine or other euphoric analgesics (cf chapter VII) There is also the possibility that the conversion of nalorphine takes place at a much slower rate in the tolerant animal than in the non-tolerant animal since the prolonged administration of morphine and codeine powerfully reduces the ability of the liver microsomes to metabolize drugs (cf JÓHANNESSON *et al* 1965 a & b) *In the course of time, numerous other hypotheses* of which only two are mentioned in detail in the present study (cf chapter V and VI) have been put forward to explain the analgesic effect and the phenomena of tolerance *Although none of these hypotheses can be said to be probable any* longer, there is yet no shadow of a doubt that they have had a stimulating effect on research, and have thus increased our knowledge of morphine and of the morphine-like substances to a considerable extent

Finally, it must nevertheless be emphasized, as was done in the introduction, that we still have an exceedingly restricted knowledge of the mechanism behind the analgesic effect and behind the *development of* tolerance Future investigations, aiming at a deeper elucidation of the me-

Chapter 3

Definitions are given of what is generally understood by tolerance and by abstinence symptoms It is emphasized that previously, tolerance to morphine was developed by administering the substance in increasing amounts, often over a period of weeks, while the results of more recent experiments show that tolerance to the analgesic effect has already developed after the administration of *relatively small doses for a brief period* It can thus be demonstrated that considerable tolerance develops to the analgesic effect of morphine and codeine after the administration of a few analgesic doses or after a single, somewhat larger dose Furthermore, tolerance to the analgesic effect of morphine is of very long duration in rats (about 1 year)

There is no support for the assumption that tolerance to morphine and codeine can be explained by a lower concentration being reached in the brain in tolerant animals The possibility is considered that tolerance may be due to reduced ability of the cells to bind morphine and similar substances in tolerant animals

Chapter 4

The *hypothesis* has been put forward that the analgesic effect of morphine is due to the formation of normorphine by N-demethylation *in vivo* It is a prerequisite of the hypothesis that normorphine itself has a strong analgesic effect More recent experiments do *not* confirm this It has also been claimed that a pronounced parallelism exists between reduced N-demethylation of morphine in the hepatic microsomal enzymes and the development of tolerance to the analgesic effect However, the results of the experiments of the author and others, show that the changes in the hepatic microsomal enzyme activity are of non specific nature, and have no relationship with tolerance to the analgesic effect

Morphine is formed *in vivo* after the administration of codeine It has been suggested, among others by the author in previous experiments that the analgesic effect of codeine may be due to bio-synthesized morphine Subsequent experiments by the author strongly suggest that the analgesic effect of codeine is *not only* due to morphine formed from codeine *in vivo* The possibility cannot be excluded, however, that morphine formed *in vivo* contributes to the analgesic effect of codeine

Chapter 5

Many investigators have claimed that the administration of cholinergic substances, such as neostigmine, for example, should have the effect of increasing the analgesic effect of morphine and similar substances However, there

the effect of codeine, while given by intraperitoneal injection it has only *2.5–5 times* the effect.

When given by subcutaneous injection morphine is absorbed directly from the subcutaneous connective tissue without passing through the liver. Morphine is inactivated to a considerable degree by conjugation to glucuronic acid in the liver. This may explain why morphine given subcutaneously has a stronger effect than morphine given by intraperitoneal injection.

Codeine is conjugated to glucuronic acid in the liver to a far lesser degree than morphine. This may explain why codeine, in contrast to morphine, has a stronger effect after intraperitoneal injection than after subcutaneous injection. The relatively slight and prolonged effect of codeine after subcutaneous injection can quite well be due to the fact that this substance is absorbed more slowly from the subcutaneous connective tissue than from the abdominal cavity.

When administered subcutaneously, morphine and codeine always *increase* each other's effect. Certain circumstances would seem to suggest that this synergism is more than a simple addition of the effects of these two substances.

Chapter 2

A brief account is given of those methods which can be used with reasonable reliability for quantitative determination of morphine and codeine in the brain. *Colorimetric* methods are known for the determination of both morphine and codeine, while morphine can also be determined by *polarography* as well as by *spectrofluorometry*. The most recent methods are based in particular on *radioactive measurements*. Using the most sensitive radioactive methods, it is possible to determine total amounts down to about 5 ng or less in pure solutions. In the case of the other methods it is not possible to carry out quantitative determinations of amounts less than 0.1–0.2 µg.

The results of three different experimental series showed that 30 minutes after intraperitoneal injection of morphine in doses which gave an analgesic reaction in 70–80 % of the rats a morphine concentration in the brain was found between about 0.2 and about 0.6 µg/g. In concurrent experiments a mean value of *180 ng morphine per g brain* was found whether the morphine was given in *equianalgesic doses* (ED_{50}) by subcutaneous injection or by intraperitoneal injection. The same concentrations in the brain were also found in experiments with equianalgesic doses of codeine whether the substance was given by subcutaneous injection or by intraperitoneal injection.

Although codeine enters the brain considerably more easily than morphine only a relatively *very small* proportion of the amounts administered is found in the brain following the administration of morphine and codeine, respectively.

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is no support for the view that the analgesic effect has a cholinergic mechanism Furthermore, in his own experiments, the author has been *unable* to confirm that neostigmine should increase the analgesic effect of morphine

Chapter 6

It has been suggested that changes in the amount of noradrenaline and serotonin in the brain play a decisive role for the analgesic effect This assumption is based to a considerable degree on experiments carried out with reserpine treated animals More recent investigations with morphine and reserpine have given exceedingly contradictory results, so that the validity of the hypothesis *is very doubtful*

Chapter 7

Although nalorphine is an antagonist to the analgesic effect of morphine, there is considerable doubt whether increasing doses of nalorphine antagonize to an increasing degree the analgesic effect of a given dose of morphine The possibility exists that nalorphine antagonizes the analgesic effect of morphine in *a manner different to that* in which it antagonizes codeine analgesia

Nalorphine can be converted to normorphine *in vivo* The possibility is discussed that normorphine may be of significance for the analgesic effect of nalorphine It is likely that morphine and nalorphine can mutually *influence each other's distribution and excretion* in dogs and rats

Chapter 8

Some general conclusions are presented, and there is a discussion of certain aspects of future research

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EJNAR MUNKSGAARD
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ANDELSBOGTRYKKERIET I ODENSE

Acta pharmacol et toxicol 1967, 25 suppl 4, 5

From the Department of Pharmacology, University of Goteborg, Göteborg Sweden

Changes in the Impulse Flow of Central Monoamine Nerves by Drugs Affecting Monoamine Receptors

By

Nils-Erik Anden

The noradrenaline and 5-hydroxytryptamine in the spinal cord are present in axon terminals of neurones the cell bodies of which are localized in the lower brain stem After transection of the spinal cord, the nerve impulses reach only the monoamine terminals cranial to but not those caudal to the lesion An inhibitor of the catecholamine or 5 hydroxytryptamine biosynthesis, produced monoamine loss throughout the intact spinal cord, but only cranial to the lesion of a sectioned spinal cord This was true for the tyrosine hydroxylase inhibitor α-methyltyrosine methylester (H 44/68) and the tryptophan hydroxylase inhibitor α-propyldopacetamide (H 22/54) Thus, H 44/68 and H 22/54 can be used to reveal changes in the impulse flow of central monoamine neurones

The adrenergic α-receptor blocking agent phenoxybenzamine and the neuroleptics haloperidol and chlorpromazine accelerated the H 44/68-induced noradrenaline depletion in the entire intact spinal cord, but only cranial to the lesion in the sectioned cord After treatment with these three drugs the catecholamine precursor dihydroxyphenylalanine did not evoke any increase in the hind limb flexor reflex in spinal rats but the formation of catecholamines was not affected Thus it is likely that the drugs block the noradrenaline receptors The adrenergic β-receptor blocking agent H 56/28 did not bring about any change in the H 44/68-induced noradrenaline depletion, or any change in the dihydroxyphenylalanine induced flexor reflex increase

Lysergic acid diethylamide (LSD) slowed down the H 22/54-induced 5-hydroxytryptamine loss in the spinal cord and in the brain Since LSD and the 5 hydroxytryptamine precursor 5-hydroxytryptophan produced similar pharmacological effects, e g on the spinal reflexes, it is possible that LSD stimulates central 5 hydroxytryptamine receptors The dopamine receptors in the corpus striatum may be stimulated in an analogous

ANDELSBOGTRYKKERIET I ODENSE

Acta pharmacol et toxicol 1967 25 suppl 4 7

From the Department of Pharmacology, University of Göteborg
Göteborg Sweden

The Influence of Nerve Impulses on the Subcellular Distribution of Metaraminol

By

Olle Almgren and Per Lundborg

Metaraminol, a noradrenaline analogue that is not attacked by monoamine oxidase or by catechol O methyl transferase, is accumulated in the adrenergic nerves and is released by nerve stimulation (ANDEN 1964, CROUT *et al* 1964) The release from the salivary glands of ³H-labelled metaraminol, given intravenously to rats in tracer doses has been studied It was found, that the amine was released by physiological nerve activity, more rapidly during the first 18 hours after the injection

The effect of nerve stimulation (continuous stimulation during 30 minutes with supramaximal stimuli, 10 impulses per second) ½ and 22-24 hours after the injection of ³H metaraminol was studied The salivary glands were treated and analyzed essentially as described by LUNDBORG & STITZEL (1967) It was found, that nerve stimulation induced a disappearance of the amine from the high speed sediment (particulate or granular fraction), while the metaraminol content of the high speed supernatant was unchanged A tendency to greater depletion at the later interval was not statistically significant

In order to investigate the intraneuronal localization of the fractions studied the retention of metaraminol in previously denervated salivary glands was studied Half an hour after the injection of the amine, only small amounts were recovered from the denervated gland After 24 hours practically nothing was left The most marked decrease occurred in the particulate fraction

Thus the amine content of the granular fraction is probably specifically reduced by nerve activity

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way by apomorphine This drug also depressed the H 44/68-induced disappearance of dopamine Both the pharmacological and biochemical effects by apomorphine were blocked by haloperidol

It is suggested that a blockade of the monoamine receptors causes an increased activity in the presynaptic neurone via a feedback mechanism consisting of several neurones The reverse action is observed after receptor stimulation

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Acta pharmacol et toxicol 1967, 25 suppl 4, 9

From the Metabolic Division, Department of Pharmacology,
University of Goteborg Sweden

Metabolic Effects of Hormones and Drugs which may be Mediated by the Adenyl Cyclos – Cyclic 3-5 AMP System

By

L. Lundholm, E. Mohme-Lundholm and N. Svedmyr

The catecholamines induce a calorigenic effect which may partly be attributed to their glycogenolytic (lactate-mobilizing) and lipolytic (FFA-mobilizing) action These effects are blocked by adrenergic β-blocking drugs As cyclic 3-5 AMP is probably a mediator of the phosphorylase and lipase activating effects of the catecholamines, we studied whether cyclic AMP itself had any calorigenic effect in the rabbit In a dose of 35–50 mg/kg, cyclic AMP increased the oxygen consumption by 20% and also increased the lactate and glucose content of the blood The FFA-content of plasma fell 5-AMP in equimolar dose had a significant less effect on oxygen consumption than cyclic AMP – The FFA-mobilizing effect of ACTH is probably also mediated via cyclic AMP In tests on the rabbit we investigated whether ACTH had any other metabolic effects which might eventually be attributable to an increased cyclic AMP formation In a dose of 25 IE, ACTH had a definitive colorigenic effect and increased the FFA and lactate content of the blood These latter effects were reduced by an adrenergic-β-blocking drug (MJ 1999)

Acta pharmacol et toxicol 1967, 25 suppl 4, 8

From the Department of Histology Karolinska Institutet Stockholm Sweden

Studies on Uptake Mechanisms in Central *Monoamine Neurones*

By

Kjell Fuxe, Tomas Hökfelt and Urban Ungerstedt

Much work has been done on the dopamine (DA) nerve terminals of the median eminence, since these terminals lie outside the blood-bran barrier These terminals have been shown to have a reserpine-resistant uptake-concentration mechanism for catecholamines (CA), which is probably fundamentally different from that found in the noradrenaline (NA) neurones Thus, this mechanism is not blocked by desipramine, protriptyline, chlorpromazine or guanethidine, but probably by cocaine Amphetamine seems to be a potent releaser of extragranular amines in the central DA neurones in the same way as in the central NA neurones In high doses 5-hydroxytryptamine (5-HT) can also be taken up by the reserpine-resistent uptake-concentration mechanism in the DA nerve terminals of the median eminence

By using intraventricular injections of 5-HT it has been possible to show that there is in the central 5-HT neurones, a reserpine-resistant uptake-concentration mechanism for 5-HT In low doses, 5-HT is selectively taken up by the 5-HT neurons in the same way as CA is taken up into the central CA neurones The reserpine-resistant accumulation of 5-HT in the 5-HT neurones was partly blocked by tryptamine and its derivatives (such as N,N-dimethyltryptamine) but not observably by desipramine, amphetamine (low doses), mescaline, or lysergic acid diethylamide Tryptamine and its derivatives were also found to decrease the amine levels of 5-HT neurones of rats treated with a combination of reserpine and nialamide – Uptake mechanisms in the central CA neurons have also been investigated using a compound which acts like α-methyl *m*-tyramine in the central nervous system i e it displaces the intraneuronal NA and to a less extent the DA stores This displacement in the NA neurons can be blocked by low doses of desipramine and particularly by protriptyline, but not by amphetamine These findings emphasise *the view* that amphetamine is a releaser of CA and not primarily a *blocker of* uptake of CA into the CA neurons

Acta pharmacol et toxicol 1967, 25 suppl 4, 9

From the Metabolic Division, Department of Pharmacology,
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Acta pharmacol et toxicol 1967, 25 suppl 4, 11

From the Department of Pharmacology University of Umeå, Sweden

Solubilization and Purification of Pig Liver Mitochondrial Monoamine Oxidase

By

G Hollunger and L. Oreland

Water washed mitochondria isolated from 0 25 M sucrose homogenate from pig liver were suspended in distilled water to a concentration corresponding to 50 mg protein per ml The suspension was extracted with ten times its volume of methyl ethyl ketone The residue, containing all the original activity was washed with buffer, suspended in 0 075 M ammonium sulphate and then once again extracted with ten times the volume of the ketone From the residue, containing about two thirds of the original activity, roughly 50% of the remaining activity was easily extracted in phosphate buffer, pH 7 2 About 80% of the phospholipids were removed by the extractions The water-clear, slightly brownish-yellow solution was then purified by gel filtration on Sephadex G-200, after concentration with $(NA_4)_2SO_4$ The purification steps resulted in a 100-fold increase in the specific activity Gel filtration on Sepharose 4 B of the purified enzyme gave a single peak appearing at the same elution volume as thyroglobulin Gel filtration on Sephadex G-200 in 1 to 2% cholate in 0 8 M $(Na_4)_2SO_4$ of the purified enzyme preparation, gave 3 new peaks with the smallest molecular weight in the region of 100 000 Copper determination of the purified enzyme showed a copper content several times less than the values reported by YASUNOBU *et al* (1966)

By all criteria the enzymes now appears to have been brought into true solution

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Acta pharmacol et toxicol 1967, 25 suppl 4, 10

From the Metabolic Devision, Department of Pharmacology, University of Göteborg Göteborg Sweden

Blockade of the Phosporylase a activating Effects of Catecholamines and Cyclic 3-5 AMP in high K^{+}- or low Na^{+} Medium

By

N. Vamos, L Lundholm and E Mohme-Lundholm

In isolated rat diaphragm suspended in a high K^{+}- buffer solution, and in which Na^{+} had been exchanged by K^{+}, the phosphorylase *a* activating effect of adrenaline and isoprenaline was blocked Substitution of Na^{+} in the solution by choline also blocked the phosphorylase *a* activating effect of adrenaline I normal Krebs-Henseleits buffer solution adrenaline increased the concentration of energy rich phosphate compounds in the diaphragm this effect was also blocked in high K^{+} The phosphorylase *a* activating effect of adrenaline is mediated by cyclic 3-5 AMP The elevating effect of adrenaline on the content of cyclic 3-5 AMP in isolated rat diaphragm was unchanged in high K^{+} The phosphorylase *a* activating effect of cyclic 3-5 AMP itself was however blocked in high K^{+} or if Na^{+} had been exchanged by choline It is suggested that the blockade of the phosphorylase *a* activating effect of the catecholamines in high K^{+} or low Na^{+} is due to an inhibition of the effect of cyclic 3-5 AMP

Adrenergic nerve fibres were studied histochemically by the formaldehyde fluorescence method according to the Falck, Owman and Hillarp method (1965) Freeze-drying was done in a copper plate chamber in a −35°C ethanol bath connected to a cold compressor and a vacuum pump for 2–3 days There was no histochemical depletion of noradrenaline in the adrenergic nerve fibres of the papillary muscle of the heart or kidney of pregnant guinea pigs, or in the spleen or kidney of pregnant rats The histochemical study of noradrenaline in the adrenergic nerve fibres in the heart huscle of pregnant rats, as well as in the spleen of pregnant guinea pigs and in the uterus of rats and guinea pigs, continues

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Acta pharmacol et toxicol 1967, 25 suppl 4, 12

From the Department of Pharmacology, Turku University, Turku Finland

The Effects of Pregnancy on the Content of Noradrenaline and Adrenaline in the Heart, Spleen and Kidney of Guinea Pigs and Rats and on the Histochemical Fluorescence Reaction of Adrenergic Nerve Fibres

By

Laes, E , Pekkarinen, A , Saarikoski, S and Suramo, I

The content of noradrenaline and adrenaline in the heart, spleen, kidneys and uterus in 13 pregnant guinea pigs and 19 pregnant rats at the end of the pregnancy and also in 13 normal female guinea pigs and 14 normal female rats, was determined by the trihydroxyindole method of v Euler and Floding (1956) modified by Pekkarinen, and the 17-OHCS content of plasma in guinea pigs by the fluorimetric method of Sweat (1954) modified by Pekkarinen and Tala The 17-OCHS mean content of plasma in the pregnant guinea pigs increased to over 7-fold, from 47 ± 9 µg% to 305 ± 29 µg%

The noradrenaline content in the heart of the guinea pig significantly decreased towards the end of pregnancy (16%) from 2 01 ± 0 10 µg/g to 1 69 ± 0 10 µg/g ($p < 0\ 05$), and also in the spleen by 56% from 0 63 ± 0 09 µg/g to 0 28 ± 0 09 µg/g ($p < 0\ 05$), but not in the kidneys The noradrenaline content of the heart muscle of rats decreased very significantly by 31% from 0 64 ± 0 04 µg to 0 44 ± 0 02 µg/g ($p < 0\ 001$), but not in the spleen or in the kidneys The decrease in the adrenaline content of the heart of guinea pigs and rats and in the spleen of guinea pigs is not due to hypertrophy, since the weight of the heart muscle, spleen and kidneys in guinea pigs and rats per 100 g of body weight during the pregnancy, is clearly smaller than in the normal control group

In agreement with the observation of Wurtman et al (1966) the noradrenaline content in the uterus decreased very significantly, 27-fold in guinea pigs, from 0 54 ± 0 09 µg/g to 0 02 ± 0 002 µg/g ($p < 0\ 001$) and 7-fold in rats, from 0 14 ± 0 01 µg/g to 0 02 ± 0 003 µg/g ($p < 0\ 001$) due

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ies did not change signi-

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From the Department of Pharmacology Turku University, Turku Finland

The Effect of Experimental Heart Insufficiency on the Content of Catecholamines in the Heart, Spleen and Kidneys and on the Fluorescence Reaction of the Adrenergic Nerve Fibres in Rats

By

Suramo, I, Saarikoski, S, Pekkarinen, A and Olli, T

The noradrenaline and adrenaline content in the heart, spleen and kidneys was determined after 1 to 16 days in 32 rats with experimental heart insufficiency with v EULER's and FLODIN's trihydroxyindole method (1956) modified by PEKKARINEN The experimental heart insufficiency was produced by constricting the ascending aorta with a silk-thread by the Chidsey method (1966) and the adrenergic nerve fibres were studied histochemically according to FALCK, OWMAN & HILLARP (1965) with the formaldehyde fluorescence method after freeze drying of a small piece of tissue for 2–3 days in a copper plate chamber connected to a vacuum pump in a -35°C ethanol bath

The normal noradrenaline content of a rat's heart, 0 67 $\pm$ 0 06 µg/g, decreased in the whole material of the heart insufficiency significantly to 0 43 $\pm$ 0 05 µg/g ($p < 0$ 01), but not in the spleen or in the kidneys The content of noradrenaline of the heart decreased most noticeable in the prolonged experimental heart insufficiencies during 6–16 days (to 0 29 $\pm$ 0 04 µg/g, $p < 0$ 001) The decrease of the noradrenaline content in a rat heart is partly due to the increase of the relative weight of heart, since in heart insufficiency the normal relative weight of the heart, 0 31 $\pm$ 0 02 g/100 g of the body weight, increased significantly to 0 39 $\pm$ 0 02 g/100 g ($p < 0$ 05), and the normal relative weight of the liver from 3 58 $\pm$ 0 13 g/100 g to 4 09 $\pm$ 0 14 g/100 g ($p < 0$ 05), but not that of the spleen and the lungs ($p < 0$ 05) The relative weight of the kidneys decreased only slightly ($p > 0$ 05) The normal adrenaline content of the spleen and the kidneys increased only slightly in heart insufficiency ($p > 0$ 05), but not in the heart

In the control rats the histochemical fluorescence reaction of the adrenergic nerve fibres was moderately strong between the muscle fibres and around the coronary arteries of the left ventricle wall in the region

of the papillary muscle, but the amount of the adrenergic nerve terminals decreased in rats with heart insufficiency as compared with the hypertrophied heart muscle fibres The histochemical fluorescence reaction decreased quite clearly in the prolonged heart insufficiencies, while, on the other hand, the fluorescence reaction of the adrenergic nerve fibre net work around the coronary arteries showed no depletion The amount and the intensity of the histochemical fluorescence reaction of adrenergic nerve fibres showed variability of depletion in individual rats with experimental heart insufficiency showing the best correlation to the duration of the experimental heart insufficiency, since a marked depletion is found in prolonged experimental heart insufficiencies The histochemical demonstration of the fluorescence reaction of the adrenergic nerve fibres of the kidneys and the spleen of the rats with heart insufficiency did not show any difference as compared with the control group

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and amitriptyline and chlorprothixene also in hemorrhagic shock in dogs (PEKKARINEN, MANNINEN & THOMASSON, 1966), although chlorprothixene diminished partially also the hypoglycaemic response in rats (MANNINEN & PEKKARINEN, 1966) As the action of acetylcholine is observed in the adrenal medulla these drugs often seem to prevent the increase of adrenomedullary secretion effectively, at the level of the adrenal medulla

Since several major tranquilizers or thymoleptic drugs are effective inhibitors of the adreno medullary secretion of insulin shock, similar inhibition might explain the adreno medullary mechanism of certain anti-emotional major tranquilizers or thymoleptic drugs

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Acta pharmacol et toxicol 1967, 25 suppl 4 16

From the Department of Pharmacology Turku University Turku Finland

The inhibiting effect of amitriptyline, bamipin and chlorprothixene on the content of adrenaline and noradrenaline in the adrenal vein of dogs and their adrenomedullary secretion caused by acetylcholine

By

Manninen, K , Pekkarinen, A and B. Thomasson

In 16 dogs, pretreated with atropine (2–3 mg/kg i m and i v) and neostigmine (0 2 mg/kg i v), large i v doses of acetylcholine (usually 0 5 mg/kg) caused a marked, but short increase of the adrenomedullary secretion in the left adrenal vein Repeated injections of the same dose of acetylcholine caused a gradual and slow decrease of this response In control dogs acetylcholine (0 5 mg/kg i v) increased the adrenaline content to 12 700 µg/l and that of noradrenaline to 5450 µg/l The corresponding secretions were 6 4 µg/kg/min and 2 7 µg/kg/min respectively in the adrenal vein The increase of the adrenaline and noradrenaline content in the adrenal vein decreased gradually after acetylcholine injections, after the 5th injection the adrenaline content was 4920 µg/l and that of noradrenaline 960 µg/l

Amitriptyline (5 mg/kg i v) in a single or repeated doses completely prevented the rise of adrenaline and noradrenaline content and secretion in the adrenal vein due to the acetylcholine injection in 6 dogs, in 4 of

rise of the adrenomedullary secretion in the adrenal vein almost completely in 4 dogs, immediately, and in addition, in one dog almost com pletely, as several repeated doses, due to the acetylcholine injection

Chlorprothixene (5–10 mg/kg i v) given as a single dose completely prevented the rise of the adrenaline and noradrenaline content and secretion in the adrenal vein in one dog and after the second or third dose almost completely in 2 dogs, while in one dog the effect was only tempo- after the first dose

in insulin shock of rats (PEKKARINEN, MAN

before the critical period and not when administered immediately afterwards Experiments with nialamide and with pargyline in combination with the monoamine precursors DOPA and 5-HTP, indicate that the inhibitory effect of pargyline is not due to increased monoamine levels

Experiments involving treatment with nialamide or nialamide-DOPA combined with hypophysectomy at different times during the critical period for LH discharge, revealed that increased monoamine levels did not advance the period of LH release, as indicated by the percentage of animals ovulating

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Acta pharmacol et toxicol 1967, 25 suppl 4, 18

From the Department of Anatomy and Brain Research Institute,
UCLA, California

Monoamines and Ovulation in the Rat

By

Bengt J. Meyerson) and Charles H. Sawyer*

Numerous pharmacologic agents have been used as tools in the investigation of neural mechanisms involved in activating the adenohypophysis to release the ovulating hormone at a definite period in proestrus (the "critical period". 14·00–16 00) in the cyclic ovulatory rat It appears that both cholinergic and monoaminergic mechanisms are involved (GAUNT *et al* 1963, SAWYER 1963; DIKSTEIN & SULMAN 1966)

The involvement of central nervous monoaminergic pathways in triggering of hypothalamo-pituitary mechanisms leading to LH release is indicated by the blockade of ovulation by reserpine when the drug is administered prior to, but not when given immediately after, the critical period of LH surge (BARRACLOUGH & SAWYER 1957) The possibility that monoaminergic pathways are involved in this neurogenic timing mechanism is also indicated by the histochemical demonstration in the median eminence at the origin of the hypophyseal portal system, of catecholaminergic nerve terminals the fibres of which arise presumably in cells of the arcuate nucleus (CARLSSON, FALK & HILLARP 1962, FUXE 1964)

In the present study it was shown that practically no animals ovulated after treatment with reserpine (5m g/kg s c) and that a *1* mg kg dose inhibited ovulation in 50% of the animals In agreement with previous data this effect was obtained only when reserpine was adminitered prior to the assumed critical period of LH release and not if the injection was given immediately afterwards

The inhibitory effect of reserpine on ovulation was prevented by treatment with either of the monoamine oxidase inhibitors, pargyline (25 mg/kg s c) or nialamide (200 mg/kg s c) Pargyline alone (50 mg/kg s c) also blocked ovulation Again this was true only if the compound was given

*) On leave from Department of Pharmacology University of Uppsala Sweden

Acta pharmacol et toxicol 1967, 25 suppl 4, 21

From the Metabolic Division, Department of Pharmacology, University of Göteborg Sweden

The Effect of Noradrenaline on the Intermediate Metabolism of Brown Adipose Tissue

By

A. Bevız and E. Mohme-Lundholm

The effect of noradrenalıne (NA) ın a concentratıon of $5 \cdot 10^{-6}$ g/ml on the metabolısm of brown adıpose tıssue from rats has been studıed In thıs concentratıon NA stımulated the oxygen consumptıon by 70%, ıncreased the formatıon of glycerol 7 tımes, doubled the FFA-release and ıntensıfied the lactate formatıon by 60% The concentratıon of hexosephosphates was changed ın that glucose-1-phosphate and fructose-1 6-phosphate were ıncreased whıle the concentratıons of glucose-6-phosphate and fructose-6-phosphate were decreased The concentratıon of "hıgh-energy" phosphate compounds (ATP + CrP) was decreased

The changes ın the hexosephosphate concentratıons was ınterpreted partly as a result of a stımulatıon of the phosphofructokınase reactıon and partly as an ındıcatıon of a phosphorylase actıvatıon The concentratıon of dıhydroxyacetonephosphate was reduced, but the glycerol-1-phosphate content, was ıncreased, also ındıcatıng an effect on the glycerol-1-phosphate dehydrogenase reactıon

After completely blockıng the FFA-mobılızıng effect of NA wıth nıcotınıc acıd, the calorıgenıc and glycerol stımulatıng effect of NA was reduced by 30% and 50% After nıcotınıc acıd, however, NA retaıned ıts decreasıng effect on the "hıgh-energy" phosphate compound content and on the stımulatıon of the lactate formatıon The lıpolytıc, calorıgenıc and glycerol stımulatıng effect of NA was totally blocked by MJ 1999 The FFA-mobılızıng effect of NA ıs therefore probably only partly responsıble for ıts calorıgenıc effect ın that ıt ıncreases the re-estrıficatıon of FFA as suggested by Ball & Jungas (1961)

The ATP-decreasıng effect of NA was assumed to be sıgnıficant for the remaınder of ıts calorıgenıc actıon and for ıts stımulatıon of the carbohydrate metabolısm

As suggested by Sutherland & Robison (1966) cylıc 3–5 AMP ıs a medıator of some of the metabolıc effects of the catecholamınes Cylıc

Acta pharmacol et toxicol 1967, 25 suppl 4, 20

From the Department of Pharmacology Karolinska Institutet
Stockholm Sweden

The effect of α- and β-adrenergic Blocking Agents on Free Fatty Acid Release from Subcutaneous Adipose Tissue *In Vivo*

By

G Bertil Fredholm and Sune Rosell

Canine inguinal subcutaneous adipose tissue was isolated from surrounding tissues After ligating all minor blood-vessels the main artery was connected to a perfusion apparatus which allowed the perfusion of the tissue at a constant rate with the dog's own blood Samples of venous blood were drawn via a drop counter into ice cold centrifuge tubes for subsequent analysis of free fatty acid (FFA) and glycerol content (ROSELL *1966a)*

Sympathetic nerve stimulation increased the release of FFA and glycerol This effect was completely blocked by β-blocking agents and considerably potentiated by α-sympatholytics The latency for the appearance of FFA into the venous blood nerve stimulation was considerably shortened after α-blockade (ROSELL 1966b, FREDHOLM & ROSELL 1967)

These effects of α-receptor blocking agents might be caused by alterations in transmitter release and/or metabolism In order to evaluate this possibility, the outflow of radioactivity into the venous blood after an infusion of DL-7-^{3}H-noradrenaline was followed Unchanged tritiated noradrenaline was separated from labelled noradrenaline metabolites by column chromatography Following nerve stimulation an increase in ^{3}H-noradrenaline outflow was seen The time course of the noradrenaline outflow was similar to that of FFA and glycerol Thus, more radioactivity appeared in the venous blood after than before α blockade The results are discussed with regard to the mechanism of action of α blocking agents on FFA and glycerol release

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From the Metabolic Division Department of Pharmacology,
University of Göteborg Sweden

The Effect of Noradrenaline on the Intermediate Metabolism of Brown Adipose Tissue

By

A. Bewz and E. Mohme-Lundholm

The effect of noradrenaline (NA) in a concentration of 5 10^{-6} g/ml on the metabolism of brown adipose tissue from rats has been studied In this concentration NA stimulated the oxygen consumption by 70%, increased the formation of glycerol 7 times, doubled the FFA-release and intensified the lactate formation by 60% The concentration of hexosephosphates was changed in that glucose-1-phosphate and fructose-1 6-phosphate were increased while the concentrations of glucose-6-phosphate and fructose-6-phosphate were decreased The concentration of "high-energy" phosphate compounds (ATP + CrP) was decreased

The changes in the hexosephosphate concentrations was interpreted partly as a result of a stimulation of the phosphofructokinase reaction and partly as an indication of a phosphorylase activation The concentration of dihydroxyacetonephosphate was reduced, but the glycerol-1-phosphate content, was increased, also indicating an effect on the glycerol-l-phosphate dehydrogenase reaction

After completely blocking the FFA-mobilizing effect of NA with nicotinic acid, the calorigenic and glycerol stimulating effect of NA was reduced by 30% and 50% After nicotinic acid, however, NA retained its decreasing effect on the "high-energy" phosphate compound content and on the stimulation of the lactate formation The lipolytic, calorigenic and glycerol stimulating effect of NA was totally blocked by MJ 1999 The FFA-mobilizing effect of NA is therefore probably only partly responsible for its calorigenic effect in that it increases the re-estrification of FFA as suggested by Ball & Jungas (1961)

The ATP-decreasing effect of NA was assumed to be significant for the remainder of its calorigenic action and for its stimulation of the carbohydrate metabolism

As suggested by Sutherland & Robison (1966) cylic 3-5 AMP is a mediator of some of the metabolic effects of the catecholamines Cylic

3-5 AMP (3 mg/ml) stimulated the O_2-consumption and the formation of glycerol and lactate These effects were not blocked by Nic ac

Theophylline (2 10^{-4} g/ml) which inhibits the enzymatic hydrolysis of cylic 3-5 AMP stimulated the O_2-consumption, and also the lactate and glycerol production The effects of theophylline was not blocked by MJ 1999 or Ni ac

In experiments on white fatty tissue – epididymal fat pads from rats – *the metabolic effects of* NA *were similar, though weaker than on brown* adipose tissue

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Acta pharmacol et tox col 1967 25 suppl 4 23

From the Department of Pharmacology Karolinska Institutet
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The Effect of Chlorpromazine on Catecholamine Synthesis in Rat and Mouse Brain

By

Henrik Nyback and Göran Sedvall

The effect of chlorpromazine on catecholamine synthesis in rat and mouse brain was determined by measuring the *in vivo* conversion of ^{14}C tyrosine to ^{14}C-catecholamines The radioactive tyrosine was adminstered to non anesthetized animals by constant rate intravenous infusion (SEDVALL, WEISE & KOPIN 1967) Following infusion the animals were killed and the brains homogenized in perchloric acid The contents of endogenous and radioactive tyrosine noradrenaline ^{14}C and dopamine ^{14}C were determined in aliquots of the extracts The catecholamines were isolated on alumina and Dowex columns Chlorpromazine (0 5–25 mg/kg) was given intraperitoneally 0 5 to 48 hours before killing the animals

Following chlorpromazine the formation of dopamine ^{14}C was increased up to three fold in both species The effect was dose dependent, it was evident within 0 5 hours after administration of the drug and lasted for about 10 hours Only in rats and only at the highest dose used was a slightly increased formation of noradrenaline-^{14}C found The specific activity of ^{14}C tyrosine was unchanged throughout the experiments

The results which support and extend those of previous studies with different techniques (CARLSSON & LINDQVIST 1963, NEFF & COSTA 1966, BURKARD GEY & PLETSCHER 1967) indicate that chlorpromazine accelerates cerebral dopamine synthesis

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Acta pharmacol et toxicol 1967, 25 suppl 4, 25

From the Department of Pharmacology, University of Göteborg, Sweden and the Department of Pharmacology, Christian Albrechts University, Kiel, W Germany

Central Hypotensive Effect of α-methyldopa

By

M. Henning and P. A. van Zwieten

In cats under chloralose anaesthesia α-methyldopa (20 mg/kg) was slowly infused into the vertebral artery One to three hours after the end of the infusion a slow but pronounced hypotensive effect was observed (mean decrease after 1 hour 17 mm Hg, s e m = 3 6, n = 9 and after 3 hours 38 mm Hg, s e m = 6 5, n = 9) The noradrenaline content of the brain was significantly lowered whereas that of the heart remained unaffected

The same low dose of α-methyldopa infused intravenously did not affect the blood pressure However, the brain noradrenaline was depleted to the same extent as observed after infusion into the vertebral artery The noradrenaline level of the heart remained normal

Infusion of saline into the vertebral artery had no effect on the blood pressure

Thus the infusion of small doses of α-methyldopa into the vertebral artery causes a clearcut fall in blood pressure Since intravenous infusion of the same low dose does not affect blood pressure, it is uncertain whether the reduction in brain noradrenaline which was observed after infusion of the drug into the vertebral artery is related to the hypotensive effect of α-mehtyldopa So far, a hypotensive effect of α-mehtyldopa in anaesthetized animals has not been described

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Acta pharmacol et toxicol 1967 25 suppl 4 27

From the Department of Pharmacology, Turku University Turku Finland

The Effect of Antihypertensive Drugs and Diuretics on the Development of Experimental Hydrocortisone and Sodium Chloride Hypertension

By

Aimo Pekkarinen and Hannu Sundqvist

Experimental hypertension was produced in rats by daily administration of hydrocortisone alcohol (2 5 mg/ml) and 2 5% sodium chloride in drinking water, according to our previous studies (LAAJOKI, KONU and PEKKARINEN 1965) Hydrocortisone alcohol is absorbed percutaneously from the tail of the rat, which is dipped once daily in the above mentioned solution Hydrocortisone alcohol and sodium chloride increased the blood pressure after 2 months to 159 ± 1 6 and 159 ± 2 1 mm Hg in two groups of 10 rats (+41 6 mm Hg above the control blood pressure) Hydrochlortiazide (10 mg/kg) frusemide (17–25 mg/kg), reserpine (3 mg/kg), Catapresan ® (0 1 mg/kg), phentolamine (12 mg/kg), guanethidine (15 mg/kg) and alpha-methyldopa (50 mg/kg) given orally very significantly ($p < 0\,001$) prevented the development of experimental hydrocortisone alcohol and sodium chloride hypertension (groups of 8–10 rats each) During the development of experimental hypertension the blood pressure was maintained at the control level during treatment with large doses of reserpine, and increased only slightly during treatment with phentolamine (+8 6 mm Hg) Catapresan ® (+9 6 mm Hg), hydrochlortiazide (+10 6 mm Hg), and alphamethyldopa (+14 6 mm Hg), guanethidine (+14 6 mm Hg) and frusemide (+19 6 and +11 6 mm Hg) for 2–2 5 months (reserpine during 2 months) above the control blood pressure In addition the increase in blood pressure slowed during drug treatment as compared with the hydrocortisone alcohol and sodium chloride groups.

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Acta pharmacol et toxicol 1967, 25 suppl 4, 26

From the Research Division of Pharmacia, Copenhagen, Denmark

Hypertensive Properties and Renal Impairment of Different Vinyl Alcohol Polymers in Rats

By

Keld Hermansen

In order to establish a hypertensive model of a pathogenesis other than that caused by DOCA pretreatment or by surgical induced renal ischemia, the hypertensive properties of different vinyl alcohol polymers have been investigated

Vinyl alcohol, (BDH), elvanol ®, (grade 71–30, du Pont) and vinol ®, (*FH1500, Air Reduction Chemical and Carbide Company, New York*) were injected subcutaneously daily to three groups of rats for a period of two months In addition saline was given ad libitum as drinking water

As a result of this treatment all three groups developed hypertension Only the vinol treatment, however, had a significant effect as compared with the saline injected control groups Vinyl alcohol (BDH) showed no nephrotoxic properties while elvanol as well as vinol impaired the renal function, as indicated by haemat- and glucoseuria, increased kidney weight and severe histological changes To check the specificity of vinol as a hypertensive agent, treatment was limited to three weeks thus decreasing the toxic manifestations of vinol This caused only a moderate hypertension

The results will be discussed with reference to the work by HALL & HALL (1963 & 1965), and the usefulness of vinyl alcohol polymers as possible tools in experimental hypertension assessed

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Acta pharmacol et toxicol 1967, 25 suppl 4 29

From the Department of Pharmacology, University of Gothenburg Sweden

Metabolic and Circulatory Effects in Man of Nicotinic Acid and an Ester of Nicotinic Acid (Pentaerythritoltetranicotinate-8AL-Bofors)

By

Nils Svedmyr

Nicotinic acid has been used clinically for more than 30 years but apart from its effect on the lipid metabolism there are few reports on its pharmacology in man Thus little is known about the relation between the concentration of this substance in the blood and its various pharmacological effects in man

A low dose of nicotinic acid (0 01 mg/kg/min) infused intravenously for 180 min raised the plasma concentration of nicotinic acid by approximately 0 4 µg/ml, i e about 50 per cent This dose caused an initial powerful trancient flush and the blood flow through the hand (skin) and the forearm (muscle) increased The pulse rate increased but the blood pressure was not altered The plasma concentration of free fatty acids decreased significantly

With a higher dose of nicotinic acid (0 05 mg/kg/min) the dilating effect on the muscle vessels was particularly greater and more prolonged.

Nicotinic acid given by mouth in a dose of one gram in conventional tablets gave a marked increase of the plasma concentration of the substance though this was of short duration At the same time there was a powerful flush and an increase in bloodflow through the hand and forearm The concentration of free fatty acids in the plasma decreased during the first three hours but thereafter increased to a level about twice the basal value

One gram of a nicotinic acid derivate pentaerythritoltetranicotinate (8 AL Bofors) given by mouth raised the plasma concentration of nicotinic acid moderately (0 6 µg/ml) At the same time the pharmacological effects mentioned above were observed though these were less marked and more prolonged

8 AL contrary to nicotinic acid gave no secondary increase of the free fatty acid content in plasma

Nicotinic acid and 8 AL were also given in enterocoated tablets which dissolved in 2 and 4 hours In this form neither of the drugs were absorbed

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From the Department of Pharmacology Karolinska Institutet
Stockholm 60, Sweden

Vascular Reactions in Canine Subcutaneous Adipose Tissue following Prostaglandin E_1 (PGE_1)

By

Bertil B. Fredholm, Bengt Öberg and Sune Rosell

PGE_1 has been shown to interfere with the lipid mobilization from adipose tissue Thus, PGE_1 inhibits the catecholamine-induced stimulation of lipolysis in rat adipose tissue *in vitro* (STEINBERG *et al* 1963) as well as in anesthetized dogs *in vivo* (BERGSTRÖM, CARLSON & ORÖ 1964)

Since the metabolic effects of PGE_1 in adipose tissue might be of physiological significance, it seemed to be of interest to study the vascular effects of this compound in some detail

A pletysmographic technique which allows analysis of the reactions of various series – coupled vascular sections was adapted to the subcutaneous adipose tissue in the inguinal region of female mongrel dogs (MELLANDER 1960, ÖBERG & ROSELL 1967)

PGE_1 was found to be a potent vasodilator agent and i a injections of 0 1 mμg, or more caused a marked rise in total blood flow PGE_1 was a more effective vasodilator agent than histamine or acetylcholine The capillary filtration coefficient (CFC) rose, indicating an increased capillary permeability and/or a rise in the number of patent capillaries PGE_1 did not have any marked effect on the venous section of the vascular bed

The results indicate that the vascular effect of PGE_1 in subcutaneous adipose tissue is mainly confined to the precapillary resistance vessels and to the exchange vessels (capillary sections)

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Acta pharmacol et toxicol 1967, 25 suppl 4 31

From the Department of Pharmacology, Karolinska Institutet,
Stockholm Sweden

Uptake and Release of Biogenic Amines in "Artificial Granules"

By

Borje Uvnäs and Karl Åborg

Mast cell granules (from rat) contain a protein heparin complex capable of binding histamine and other biogenic amines The amines are probably linked to weak acidic groups of the complex and not to the sulphate groups of heparin, as previously assumed Whether the weak acidic groups are localized in the protein or in the heparin is unknown

The protein of the protein heparin complex in the granules is reported to be alkaline with a relatively high arginine content We have studied the amine binding properties of an *in vitro* complex between heparin and protamine – an alkaline protein with a high arginine content

The observations demonstrated close similarities in the amine binding properties between mast cell granules and the protamine-heparin complex (artificial granules) Quantitative calculations indicate the amines to be linked to carboxylic groups of the protamine part of the protamine-heparin complex

Acta pharmacol et toxicol 1967, 25 suppl 4 30

From the Department of Pharmacology University of Göteborg Sweden

Threo-α-Methyl Noradrenaline: A Study on Uptake, Release and Metabolism

By

Bertil Waldeck

^{3}H-labelled (±)-threo α-methylnoradrenaline was injected intravenously into mice The amine was rapidly taken up by the heart and then disappeared slowly In animals pre-treated with reserpine there was still an uptake, but the amine disappeared more rapidly Protriptyline pretreatment blocked the uptake almost completely In comparison with erythro ^{3}H-α-methylnoradrenaline, the threo ^{3}H-α-methylnoradrenaline which had accumulated in the heart of untreated animals, showed a more rapid disappearance at a long time interval

Reserpine given after threo ^{3}H-α-methylnoradrenaline caused a release of the amine, whereas protriptyline did not do so In combination with reserpine, protriptyline appeared to potentiate slightly the releasing effect of reserpine

Ion-exchange chromatography of a femoral muscle extract revealed a metabolite of threo ^{3}H-α-methylnoradrenaline which was considered to be threo ^{3}H-α-methylnormetanephrine This was also demonstrated by blocking catechol-O-methyl transferase by α-propyldopacetamide

The data reported here are discussed in view of previous data on the two diastereoisomers of α-methylnoradrenaline It was concluded that, in spite of a much weaker receptor affinity of threo α-methylnoradrenaline, both isomers have essentially the same relation to the uptake and storage mechanisms of the adrenergic neuron

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Acta pharmacol et toxicol 1967, 25 suppl 4 32

From the Department of Pharmacology, Karolinska Institutet
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Uptake of Biogenic Amines by Mast Cell Granules

By

Anders Bergendorff and Borje Uvnäs

Biologically active substances, for example histamine and heparin, are localised in the mast cells, where they are found in the granular fraction The rat mast cell also contains 5-hydroxytryptamine Granules from rat mast cells have been shown to be very useful in studies on release and uptake mechanisms since they are relatively easy to isolate and furthermore are very slightly soluble in water and isotonic salt solutions The granules consist of a heparin-protein complex to which histamine is bound

The uptake and release mechanism for histamine has been shown to be pH-dependent and it has also been shown that cations are capable of replacing histamine These results suggest that the binding between histamine and the heparin-protein complex is electrostatic and that it is probably a binding to weak acid groups in the complex One could perhaps compare the granules to a cationexchanger of the carboxylic type

A recent investigation on the specificity of the binding has shown that other amines such as 5-hydroxytryptamine, adrenaline, noradrenaline, dopamine, tyramine, phenylethylamine and acetylcholine can be taken up by the granules Thus the linkage between histamine and the granules does not seem to be specific There are certain differences however in the affinities of the different amines for the granular binding sites The uptake of the other amines also seem to be pH-dependent and it is possible to release them with sodium Therefore one would expect the binding between the amines investigated and the heparin-protein complex to be the same as for the naturally occurring histamine

Acta pharmacol et toxicol 1967, 25 suppl 4 33

From the Department of Pharmacology Karolinska Institutet
Stockholm, Sweden

The Mechanism of Histamine Release from Rat Mast Cells Induced by Adenosine Triphosphate

By

Bertil Diamant

Histamine release from isolated rat mast cells is induced when the cells are incubated with ATP (DIAMANT & KRUGER 1967) The effective concentration (2–4 × 10^{-5} M) is well below the intracellular concentration of ATP (4 × 10^{-4} M) (DIAMANT 1967)

Among the organic adenosine phosphoresters, the histamine releasing activity is specific for ATP while ADP, AMP, 3′5′AMP are not effective even in concentrations around 10^{-3} M Adenosine, phosphoenolpyruvate and phosphocreatin are also without effect

When isolated rat mast cells are incubated with ATP, hydrolysis of the ATP occurs This "ATP-ase" activity of rat mast cells seems to be the same for intact cells and for broken up cells, indicating the nature of an "ecto-ATP-ase"

When the ATP-hydrolysis and the ADP-formation was followed in the same preparation only 25–50% of the hydrolysed ATP could be accounted for as ADP The apparent reason for this is that the mast cells also hydrolyse ADP ADP inhibits hydrolysis of ATP by the mast cells as well as histamine release induced by ATP The finding that Ca^{++} is a co-factor for the enzyme points to an apyrase like character of the enzyme Since ATP lacks histamine releasing activity in the absence of Ca^{++} it seems possible that this ATP-"ecto apyrase" system constitutes a new mechanism for histamine release that might have physiological implications Some possible effects of this system on the mast cells will be discussed

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Diamant B The [illegible]
phate content [illegible] press

Acta pharmacol et toxicol 1967, 25 suppl 4, 34

From the Department of Pharmacology, University of Umeå Sweden

Respiration of Mast Cells during Histamine Release

By

Nirmal Chakravarty

The respiration rate of a pure population of rat peritoneal mast cells was measured in silicone-coated ampulla divers of about 10 µl gas volume The antigen solution (egg albumin) was placed as a drop on the wall of the diver close to the mast cells, which were actively sensitized to the antigen After an initial record of the basal respiration rate at 37°, the cells were mixed with the antigen without removing the diver from the bath and the respiration measurement was continued Immediately after contact with the antigen – which triggered the histamine release – there was about 30% stimulation of oxygen uptake The stimulation persisted for 15–20 minutes, after which the respiration rate returned to the basal level This effect was characteristically seen in the absence of a substrate in the medium In the presence of glucose the stimulation was very mild Control experiments in which unsensitized cells were mixed with a drop of the medium or of the antigen solution, did not show any increase in oxygen uptake

It is now well known that anaphylactic histamine release can be blocked by lack of oxygen The role of oxygen is not however clearly understood The present finding is consistent with the assumption that oxidative energy metabolism is directly involved in the release process

Acta pharmacol et toxicol 1967, 25 suppl 4, 35

From the Department of Pharmacology, University of Umeå Sweden

Inhibition of Histamine Release from Rat Mast Cells by 2-deoxyglucose

By

Nirmal Chakravarty

2-deoxyglucose (2 DG) readily inhibits the anaphylactic histamine release from guinea pig tissue but does not prevent the compound 48/80-induced release from rat tissue in an aerobic medium Since the mechanisms of these release processes seem to be very similar, an attempt has been made in the following experiments to find out if the difference in the effect of 2-DG on histamine release from guinea pig and rat tissues can be explained within the general frame of metabolic inhibition caused by 2-DG in different tissues The effect of 2-DG was studied on histamine release from rat peritoneal mast cells induced by compound 48/80 or antigen-antibody reaction *in vitro* In an oxygenated medium 2-DG had no effect on compound 48/80-induced histamine release and inhibited anaphylactic release only in high concentrations Acetate and pyruvate – unlike their effect on guinea pig tissue – could not reverse the 2-DG inhibition of anaphylactic histamine release from rat mast cells Cyanide inhibition of both compound 48/80-induced and anaphylactic histamine release was potentiated by a low concentration (5 mM) of 2-DG When glucose was used to restore histamine release blocked by cyanide, 2-DG (5 mM) markedly inhibited the release Iodoacetate, fluoride and oxamate had a similar blocking effect on the glucose-sustained histamine release in the presence of cyanide The findings are explained on the assumption that 2-DG mainly acts in rat mast cells by blocking the glycolytic pathway and that the oxidative hexose metabolism in rat mast cells – unlike that in guinea pig tissue – is relatively insensitive to 2-DG

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Acta pharmacol et toxicol 1967, 25 suppl 4 34

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Acta pharmacol et toxicol 1967, 25 suppl 4 36

From the Department of Pharmacology, University of Copenhagen

Influence of Anti-rheumatic Agents on the Release of Histamine from sensitized Rat Peritoneal Mast Cells

By

Svend Norn

The liberation of histamine from rat peritoneal cells caused by an antigen – antibody reaction can be inhibited by treating the rats with various anti-rheumatic agents (Norn 1965) The investigator elucidated the following mechanisms for inhibiting the histamine release:

1) Rats were sensitized passively by the intraperitoneal route with dialysed serum from sensitized rats untreated with anti-rheumatic agent (control) or treated during the last 3 days of the sensitization period with a daily subcutaneous injection of 100 mg per kg hydrocortisone, 25 mg/kg sodium aurothiosulphate or 200 mg/kg phenylbutazone The peritoneal cell suspensions from the passively sensitized rats were then incubated *in vitro* with antigen No significant difference was obtained in histamine release This indicates no decrease in serum – antibody concentration in the donors treated with antirheumatic agents, i e, the anti-rheumatic agents cannot have caused inhibition of antibody production

2) Peritoneal cells from rats, untreated (control) or treated with anti-rheumatic agents as mentioned above, were injected intraperitoneally into sensitized rats depleted of peritoneal mast cells by previous injections of water After passive sensitization of the transferred cells, they were incubated *in vitro* with antigen No significant difference was found in histamine release Therefore the anti-rheumatic agents cannot have influenced the mast cells or the enzymatic processes which are assumed to be elicited in the mast cell during antigen – antibody reactions

3) Peritoneal cells from sensitized rats were pre-incubated with anti-rheumatic agents and then incubated with antigen Hydrocortisone and phenylbutazone but not sodium aurothiosulphate (respectively 200, 100 and 1000 µg/ml cell suspension) inhibited the histamine release The inhibiting- mechanism for hydrocortisone and phenylbutazone may therefore be due to an inhibited antigen – antibody reaction

Acta pharmacol et toxicol 1967, 25 suppl 4 39

From the Department of Pharmacology and Toxicology, Veterinary College of Norway, Oslo

^{51}Cr-EDTA as a Reference Substance in Research on Gastrointestinal Functions

By

Per Lökken and Erling Sognen

^{51}Cr-EDTA (Tetraceminum NFN) represents an incorporation of the analytical advantages of the γ ray emitting ^{51}Cr-isotope in a water-soluble highly stable chelated compound

After oral administration of ^{51}Cr-EDTA to intact rats about 5% of the activity was absorbed Following parenteral injection, there was a rapid and complete discharge of activity from all organs Approximately 50% appeared in the urine within one hour

Self-curing acrylic material was used to fix a urine collecting device to rats The arrangement allowed of separate quantitative collection of urine and feces from conscious animals for a period up to 3–4 days

About 8% of the complex disappeared from the ligated duodenum of rats in one hour, less from the jejunum, while the absorption from the stomach and the ileum was insignificant

During perfusion of the small intestine *in situ*, the disappearance rate of ^{51}Cr EDTA was comparable to that of phenol red, i e about 5% in 30 minutes

^{51}Cr-EDTA proved to be unsuitable as a reference substance in everted intestinal loops

Compared with ^{51}Cr EDTA the gastrointestinal passage of phenol red in intact animals was delayed This retarded passage may erroneously indicate absorption

Using ^{51}Cr EDTA as a reference substance we are at present studying the interrelationship between drug induced changes in gastrointestinal functions and drug absorption Despite some absorption, we have found ^{51}Cr EDTA preferable for tracing the flow, absorption and dilution of [illegible]

[illegible]

[illegible] clearance

Acta pharmacol et toxicol 1967, 25 suppl 4, 38

From the Department of Pharmacology, Karolinska institutet, Stockholm Sweden

Release of Histamine and Spasmogenic Lipids from Guinea-pig Lung Tissue Induced by Phosphatidase A and Antigen

By

B. Fredholm and K. Strandberg

Incubation of chopped guinea-pig lung tissue with bee venom leads to the appearance of histamine and spasmogenic lipids in the incubation fluid The histamine releasing effect of the venom has been ascribed to its content of phosphatidase A Studies with bee venom, fractionated by gel filtration, have shown that in the rat, histamine release from peritoneal mast cells is not induced by the phosphatidase A fraction, F I, but by another fraction, F II (FREDHOLM & HAEGERMARK 1967) To ascertain which bee venom fraction is active in guinea-pig lung, chopped lung tissue from non-sensitized and sensitized guinea pigs was incubated with bee venom, F I and F II Unfractionated bee venom, as well as F I, induced release of histamine and the appearance of spasmogenic lipids, while F II had no such effect

Various aspects of the F I-induced release were studied and compared with the results obtained with antigen, namely the dose-response relation, the time course, the temperature dependency, and the influence of anoxia Both with regard to histamine release and the appearance of spasmogenic lipids, F I was found to act in a manner different from that of antigen The release did not seem to involve an energy-requiring mechanism in the tissue Comparing the spasmogenic lipids obtained by incubation with F I and antigen, respectively, no differences were found in the biological or chemical properties tested

On incubation with F I, the appearance of spasmogenic lipids preceded the release of histamine This is in contrast to the findings with antigen and seems to indicate that the involvement of mast cells is not essential for the phosphatidase A-induced formation of spasmogenic lipids

It is concluded that in guinea-pig lung the phosphatidase A containing fraction of bee venom (F I) induces both release of histamine and formation of spasmogenic lipids by mechanisms at least in part different from those involved in the antigen-antibody reaction

REFERENCE

Acta pharmacol et toxicol 1967, 25 suppl 4 41

From the Department of Pharmacology, Royal Veterinary College, Stockholm, Sweden

Factors Affecting the Uptake, Elimination and Toxicity of Nicotine in the Mouse

By

Torbjorn Stålhandske

Acquired tolerance against the toxic effects of nicotine may either be due to an increased capacity of the body to metabolize the substance and/or to adaptation

Nicotine is mainly metabolized in the liver and also to a small extent, in the kidneys and the lungs, but not in the mouse brain It has been proposed that animals tolerant to the substance may have an increased metabolism of nicotine in both liver, lungs and kidneys This increase however is not very large and not significant

Pretreatment with phenobarbital increases the liver metabolism of a number of pharmacological substances In the present investigation pretreatment with phenobarbital significantly increased the *in vitro* metabolism of nicotine Animals pre treated with phenobarbital also had an LD50-value about three times higher when nicotine was given intraperitoneally However, when nicotine was given intravenously the LD50-value did not differ from the controls Pre treated animals also had a greater tolerance to repeated intraperitoneal sublethal doses of nicotine

Quantitative estimations of the concentration and elimination of C^{14} nicotine in brain, liver and blood both after intravenous and intraperitoneal injections showed that phenobarbital treated animals had a much more rapid elimination of nicotine from the liver This seemed to affect the nicotine concentration in the brain after intraperitoneal injection In both the liver and the brain, the concentration was about three times lower in pretreated animals after an intraperitoneal injection However, when nicotine was given intravenously the phenobarbital induced increase in liver metabolism of nicotine did not affect the concentration in the brain The toxic effects of nicotine are mainly due to an action on the central nervous system and the effects are very likely related to the concentration in brain This explains why phenobarbital pre treated animals have a greater tolerance to lethal and sublethal doses of nicotine given intraperitoneally and why it is impossible to produce tolerance to intravenous injections of nicotine when only the breakdown of nicotine is increased Increased metabolism of nicotine may thus produce tolerance to nicotine except when it is given directly into the blood

Acta pharmacol et toxicol 1967, 25 suppl 4, 40

From the Pharmacological laboratory, Leo Pharmaceutical Products Ballerup Denmark

The Influence of Ethanol on the Absorption of Drugs from the Rat Small Intestine

By

M P. Magnussen

Particularly in recent years, the alcohol – drug interactions have become a progressively serious problem in forensic as well as in social medicine

In previous experiments (MAGNUSSEN & FREY 1967), performed on rats under urethane anaesthesia, alcohol significantly enhanced the absorption from the stomach of drugs which at the pH of the stomach were present in their uncharged diffusible form This effect was shown to be the consequence of an enhanced blood circulation in the gastric mucosa under the influence of alcohol

As most of the drug absorption takes place from the small intestine, it seemed worth-while finding out whether alcohol at this site had the same effect In urethane anaesthesia, the absorption from the rat small intestine was measured by means of the perfusion method described by SCHANKER *et al* (1958) In one series of experiments the small intestine was perfused with purely aqueous or alcoholic (0 5, 1 0, 2 0%) drug solutions Experiments were made with barbitone, phenobarbitone, pentobarbitone, promethazine and sulphaguanidine, and in no case did alcohol have any effect on the absorption In another series of experiments a steady state of blood alcohol concentration of 1 or 1 5 mg/ml was shown to have no effect on the absorption of barbitone and promethazine

The absence of effect of alcohol on the intestinal absorption of drugs is considered to be the consequence of the enourmous absorbing area, thus providing optimal conditions that can no longer be changed by relatively small variations in the blood supply

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Acta pharmacol et toxicol 1967, 25 suppl 4 41

From the Department of Pharmacology, Royal Veterinary College, Stockholm Sweden

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Acta pharmacol et toxicol 1967, 25 suppl 4, 42

From the Pharmacological Laboratory, Alfred Benzon Ltd, Copenhagen V, Denmark

Plasma Concentrations of Monophenylbutazone, Phenylbutazone and Ketophenylbutazone after Intravenous Administration to Rats and Mice

By

G. Hegermann Nielsen and H. Holmen-Christensen

A comparative study was performed on three pyrazolidinederivatives regarding the rate of their elimination from the blood stream in rats and mice

Determination of the plasma levels was carried out using a new method of analysis, the main points of which are a thin-layer chromatographic isolation followed by an UV-spectrophotometric analysis of the substances dissolved in alcohol

This method is valuable because it may avoid problems liable to arise from an uncontrolled interference of the metabolites, which in these species may appear as soon as $\frac{1}{4}$–$\frac{1}{2}$ hour after injection

Blood samples were drawn by heart-puncture (ether anaesthesia) at increasing intervals from 3 minutes to 6 hours after injection, each animal, however, being used only once

From the graphs showing plasma levels related to time it appears that by far the major part of the amount injected of each compound was eliminated within one hour of administration From then on the rate of elimination decreased more or less, depending on both the quality and quantity of the substance

The result of the study emphasizes the difficulty of expressing the biological half-life of at least this type of drug by one single figure

Acta pharmacol et toxicol 1967, 25 suppl 4 43

From the Department of Pharmacology University of Copenhagen

The Effect of Anti-rheumatic Drugs on the Protein Binding of Cortisol in Plasma and the Cortisol Content in Connective Tissue

By

Jens Aas Jansen and Jens Schou

In the guinea pig, as in man cortisol is the main adreno-glucocorticoid Guinea pigs were *therefore chosen for the investigation* *They were* pretreated for 3 days with two daily injections of phenylbutazone (48 mg/kg/day), sodium salicylate (800 mg/kg/day) indomethazin (16 mg/kg/day) while the controls were injected with saline

Two hours after the last injection, blood was sampled by heart puncture and a depilated skin sample was obtained from the back

The content of cortisol was determined by a spectrophotofluorometric micromethod (JANSEN, HVIDBERG & SCHOU 1967) in plasma, in ultrafiltrate of plasma and in the skin sample

The *cortisol concentration in plasma* (ng/ml, mean of 12 experiments) for controls was 244 and was unchanged by indomethazine treatment Salicylate increased (280) and phenylbutazone decreased (197) the plasma cortisol

The *concentration of free (unbound) cortisol* seemed to vary in parallel with the total plasma concentration Approximately 28% of the total plasma cortisol was found in the ultrafiltrate Pretreatment with the anti rheumatic agents mentioned did not seem to influence the protein binding of cortisol in plasma

The *concentration of cortisol in skin* connective tissue also varied in parallel with the plasma (free and bound) cortisol The highest values were found in the salicylate treated group (87 ng/g, n = 12), and the lowest values in the phenylbutazone treated group (42 ng/g)

The investigation does not support the theory that non-steroid antirheumatic agents exert their effect by altering the ratio between free and bound cortisol in the plasma

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This method is valuable because it may avoid problems liable to arise from an uncontrolled interference of the metabolites, which in these species may appear as soon as ¼–½ hour after injection

Blood samples were drawn by heart-puncture (ether anaesthesia) at increasing intervals from 3 minutes to 6 hours after injection, each animal, however, being used only once

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The result of the study emphasizes the difficulty of expressing the biological half-life of at least this type of drug by one single figure

Acta pharmacol et toxicol 1967, 25 suppl 4 45

From the Department of Biochemistry A University of Copenhagen, Copenhagen, Denmark

The Influence of L-Thyroxine on the Metabolism of Ethanol in Rat Liver

By

A K. Rawat and P. Schambye

The metabolism of ethanol involves oxidation to acetaldehyde followed by conversion to acetate The hydrogen is transferred to NAD^+ resulting in an increase in the $NADH/NAD^+$ *) ratio in the liver with a concomitant increase in the concentration of lactate and L-glycerophosphate

In rats treated with thyroxine the mitochondrial L-glycerophosphate dehydrogenase activity in the liver is increased and the concentration of L-glycerophosphate decreased It was therefore decided to study the rate of ethanol oxidation in the livers of rats treated with L-thyroxine Liver slices from treated and control rats were incubated in Krebs-Ringer bicarbonate buffer for 60 minutes in the presence of ethanol (4 mM) Under these conditions the average rate of ethanol uptake was 41 4 $\pm$ 5 7 µmoles/g/hr in the controls and 35 8 $\pm$ 4 4 µmoles/g/hr in the treated rats This difference is not statistically significant The $NADH/NAD^+$ ratio, measured as lactate/pyruvate ratio, also did not change, although the average lactate release decreased from 18 4 to 8 4 µmoles/g/hr

The inhibition of glycerol uptake produced by ethanol in liver slices (THIEDEN, unpublished) was abolished in livers from hyperthyroid rats, as the uptake was from 33 1 to 43 2 µmoles/g/hr in the presence and from 30 0 to 40 7 µmoles/g/hr in the absence of ethanol The increase in L-glycerophosphate concentrations in the presence of ethanol was not observed in liver slices from thyroxine treated animals

Inspite of the increased oxidation of L-glycerophosphate to dihydroxyacetonephosphate occurring in the hyperthyroid animals, no increase in ethanol uptake was observed, nor did the presence of glycerol up to 20 mM increase the ethanol uptake of the hyperthyroid livers This may indicate that NAD^+, acting as hydrogen acceptor in the ethanol oxidation, was not regenerated by the reduction of dihydroxyacetonephosphate

*) NADH and NAD^+ are the reduced and oxidised nicotinamide adenine dinucleotides, respectively

Acknowledgement

The investigation was supported by the Danish State Research Foundation

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Acta pharmacol et toxicol 1967, 25 suppl 4, 45

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Acta pharmacol et toxicol 1967, 25 suppl 4, 46

From the Department of Pharmacology, University of
Copenhagen, Denmark

Decamethonium Uptake in the Liver of Intact Mice and by Slices of Mouse Liver

By

Chr. Broen Christensen

Mice were given 0 15 µg/g ^{3}H-decamethonium intravenously and the distribution of decamethonium was studied by measuring the radioactivity in plasma, striated muscles and liver The experiments revealed a rapid fall of radioactivity in the blood 20 minutes after the injection the striated muscles had taken up only 14% of the dose, whereas 50% of the dose was found in the liver Radioactivity extracted from liver corresponded chromatographically to decamethonium

The decamethonium uptake by liver tissue was examined more closely *in vitro* Liver slices were incubated in Krebs-Ringer-bicarbonate medium containing decamethonium (2 × 10 ^{6}M) and the slice-to-medium concentration ratio was measured The results suggest that decamethonium is taken up by a process showing several of the characteristics of active transport Further, D-tubocurarine (2 × 10 ^{4}M) almost completely inhibited the uptake

Acta pharmacol et toxicol 1967, 25 suppl 4 47

From the Department of Pharmacology, University of Göteborg
Göteborg Sweden

Alteration in Subcellular Distribution of Amines Produced by Drugs

By

P. Lundborg and R. Stitzel

Most of the previous studies on adrenergic nerve granules have been performed *in vitro* Thus it was found of interest to perform experiments in which all processes studied were carried out *in vivo*

Investigations of this kind are difficult to perform with NA since the extragranular fraction of NA is rapidly broken down by MAO

Two possible ways of performing experiments of this kind are discussed 1) ^{3}H-NA is administered after MAO-inhibition 2) α-methylated amines like α-MeNA or metaraminol are used

Both *in vivo* and *in vitro* reserpine is a potent inhibitor of the uptake of ^{3}H-NA and ^{3}H-α-MeNA into the amine storage granules (particulate fraction) After reserpine pretreatment of mice there is a marked block of the uptake of administered ^{3}H-NA and ^{3}H-α-MeNA into the granules with subsequent extragranular accumulation of the amines The accumulation of ^{3}H-NA can be demonstrated only after pretreatment with a MAO-inhibitor (Nialamide)

The releasing effect of drugs on previously administered ^{3}H-metaraminol was studied Reserpine selectively released ^{3}H-metaraminol from the particulate fraction and protriptyline from the supernatant fraction Reserpine was more effective at long intervals after ^{3}H-metaraminol injection while protriptyline was effective only at short intervals The interpretation for this is that there is a transfer of ^{3}H-metaraminol from one granular pool to another

It is suggested that the models used in these experiments could be used for distinguishing between different sites of action of drugs interfering with amine storage and release

Acta pharmacol et toxicol 1967, 25 suppl 4 46

From the Department of Pharmacology, University of Copenhagen, Denmark

Decamethonium Uptake in the Liver of Intact Mice and by Slices of Mouse Liver

By

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Acta pharmacol et toxicol 1967, 25 suppl 4, 49

From AB Leo Research Laboratories, Helsingborg, Sweden

Distribution and Excretion of Labelled 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphtalane (Lu 3-010) in the Rat and Dog

By

G. Plym Forshell

Tritium-labelled Lu 3-010 was made according to Wilzbach The distribution of the compound in rats was followed during one week The compound was given intravenously in a dose of 5 mg/kg During the first 8 hours the greatest concentration of radioactivity was found in the lungs and the liver The concentration in the brain was the same as the concentration in the blood and fat Maximum excretion rate in the urine was obtained during the first hour and the excretion ceased within 48 hours Equal amounts of radioactivity were excreted in urine and faeces

Dogs were given 1 mg/kg intravenously of ^{14}C-labelled Lu 3-010 The distribution pattern was the same as in the rats The excretion of the drug was followed in two dogs with a bile fistula No radioactivity was recovered in the bile but 35% of the administered dose was found in the faeces These findings were confirmed in experiments with anaesthetized dogs, where the bile was collected directly from the gallbladder. The results indicate that the substance is partly excreted by the secretory tissues in the stomach, pancreas and salivary glands

Analysis of extracts from urine, faeces and tissues was done by thin-layer chromatography

Acta pharmacol et toxicol 1967, 25 suppl 4, 48

From the Research Laboratories of H Lundbeck & Co, A/S,
Ottiliavej 7, Copenhagen Valby, Denmark

Metabolism of 3,3-dimethyl-1-(3-methyl-aminopropyl)-1-phenylphthalane (Lu 3-010), a Bicyclic Compound with Thymoleptic Properties

By

Aksel Jørgensen and Ulla Dahl Larsen

The metabolism of 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalane (Lu 3-010) has been investigated by means of a chromatographic analysis of urine collected from dogs, given an oral dose of 25 mg/kg daily for six months

In addition to the unchanged substance two metabolites were found in considerable amounts, namely the primary amine corresponding to the mother substance, and the acid formed by oxidative deamination of this amine In rats, which excrete the same metabolites in the urine as dogs, it is possible to reduce considerably the amount of the acid metabolite in the urine by treatment with a MAO-inhibitor, a fact which indicates that the oxidative deamination is catalysed by mono-amineoxidase Most remarkable is the appearance of considerable amounts of an acid metabolite (formed by demethylation and oxidative deamination of the mother substance) A corresponding metabolite is also known from other psychotropic drugs, e g chlorpromazine, but in this case appears only in insignificant amounts No phenolic metabolites were detected

Acta pharmacol et toxicol 1967 25 suppl 4 49

From AB Leo Research Laboratories Helsingborg Sweden

Distribution and Excretion of Labelled 3,3-dimethyl-1-(3-methyl-aminopropyl)-1-phenylphtalane (Lu 3-010) in the Rat and Dog

By

G Plym Forshell

Tritium labelled Lu 3-010 was made according to Wilzbach The distribution of the compound in rats was followed during one week The compound was given intravenously in a dose of 5 mg/kg During the first 8 hours the greatest concentration of radioactivity was found in the lungs and the liver The concentration in the brain was the same as the concentration in the blood and fat Maximum excretion rate in the urine was obtained during the first hour and the excretion ceased within 48 hours Equal amounts of radioactivity were excreted in urine and faeces

Dogs were given 1 mg/kg intravenously of ^{14}C-labelled Lu 3-010 The distribution pattern was the same as in the rats The excretion of the drug was followed in two dogs with a bile fistula No radioactivity was recovered in the bile but 35% of the administered dose was found in the faeces These findings were confirmed in experiments with anaesthetized dogs where the bile was collected directly from the gallbladder The results indicate that the substance is partly excreted by the secretory tissues in the stomach, pancreas and salivary glands

Analysis of extracts from urine, faeces and tissues was done by thin layer chromatography

Acta pharmacol et toxicol 1967, 25 suppl 4, 50

From the Pharmacological Laboratory, Alfred Benzon Ltd, Copenhagen V, Denmark

A Comparative Study on the Excretion and Metabolic Degradation of Nitrofurantoin and Analogues

By

H. Holmen-Christensen and H. O. Andersen

Nitrofurantoin and Hydroxy-methyl-nitrofurantoin were given orally both to laboratory animals and human subjects The excretion and metabolic degradation in the urine and blood was studied

A new method is described for the determination of Nitrofurantoin and Hydroxy-methyl-nitrofurantoin in the urine and blood By this method a hitherto unknown metabolite of Nitrofurantoin was found, and separated from the parent compound by thin-layer chromatography

Comparison was made between our own results and those obtained by the colorimetric method of BUZARD, and by determining the microbiological activity according to JONES and STEVENS, respectively

A comparative evaluation of the methods mentioned is made and conclusions concerning the therapeutic use are drawn

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Acta pharmacol et toxicol 1967, 25 suppl 4 51

From the Institute of Pharmacology University of Oslo Norway

Inhibition of the Renal Tubular Transport of p-aminohippurate-(Tm-PAH) in the Rabbit caused by Sub-toxic Doses of Dichlorophenoxyacetate (2,4-D)

By

Fred Dybing and Andreas Kolberg

NIELSEN, KÆMPE & JENSEN HOLM in 1964 observed higher concentrations of 2,4-D in the blood than in the urine of a case of suicide with this herbicide

One possible explanation is that there is active reabsorption of 2,4-D by the renal, tubular epithelium If so, it would be expected that 2,4-D also had an effect on the transport capacity of the tubular epithelium for organic acids e g p-aminohippurate

Four rabbits, weighing 3 6–4 3 kg were assayed for Tm-PAH and C-Cr (glomerular filtration rate expressed as exogenous creatinine clearance)

There were eleven clearance periods for control and 13 periods during continuous intravenous infusion of 2,4-D, 2 mg/min Total 2,4-D dose per animal varied between 33 and 69 mg/kg because of different durations and numbers of clearance periods

Tm PAH fell from the control mean of 8 4 to 6 5 mg/min – hardly statistically significant ($p < 0\ 05$) C-Cr increased insignificantly from 10 1 to 11 7 ml/min The Tm PAH/C-Cr ratio fell significantly from 0 87 to 0 55 ($p < 0\ 01$)

Another 4 rabbits weighing 3 3 to 4 3 kg received priming intravenous injections of 100 mg 2,4-D, followed by a sustaining infusion of 2 mg/min so that total doses per animal were 62–115 mg/kg

Twelve control periods and 13 with 2,4-D were run

Tm PAH decreased from 8 0 to 2 9 mg/min and the Tm-PAH/C-Cr ratio fell from 0 91 to 0 44 with unaltered glomerular filtration rates These decreases are both highly significant

No clinical signs of intoxication were observed and the general condition of the animals remained unaltered both during the experiments and during the following few days

Acta pharmacol et toxicol 1967, 25 suppl 4 50

From the Pharmacological Laboratory, Alfred Benzon Ltd, Copenhagen V, Denmark

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Paul and Paul The Nitrofurans – Chemotherapeutic Properties *Exp Chemother* 1964 II 307–70

Acta pharmacol et toxicol 1967, 25 suppl 4, 53

From the Department of Physiology, University of Reykjavik, Iceland

Effects of Caffeine on Electrical and Mechanical Activity of Smooth Muscle

By

J. Axelsson and G. Hogberg

Caffeine activates the contractile elements in depolarized and normally polarized striated muscle The effect on tension is mediated without any changes in membrane potential or resistance (AXELSSON & THESLEFF 1958)

We have studied the effect of caffeine on the electrical and mechanical activity of intestinal smooth muscle, the taenia coli from guinea-pig, and vascular smooth muscle, the portal vein of the rat Caffeine caused an increase in the spontaneously maintained tone of taenia coli In isometric conditions at 110% L_0 (see ÅBERG & AXELSSON 1965) the increase in tension was explained by an increase in spike amplitude and frequency of discharge causing summation and fusion of individual tension responses Contractures maintained by depolarized muscles were however relaxed by caffeine Furthermore, caffeine decreased both the maintenance and amplitude of the mechanical responses to supramaximal stimuli

In the portal vein spontaneous activity consists of bursts of spikes at intervals closely followed by tension development (AXELSSON *et al* 1966) Caffeine increased the frequency of contractions but decreased their amplitude These changes were adequately explained by changes in the pattern of electrical activity

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Acta pharmacol et toxicol 1967, 25 suppl 4, 52

From the Research Laboratories of AB Astra, Södertalje, Sweden, and the Laboratory of Biological Research, Astra Pharmaceutical Products, Inc, Worcester, Mass USA

Studies with Optical Antipodes of Compounds Possessing Local Anaesthetic Activity

By

B. Åkerman, G. Camougis and R. Sandberg

Differences in local anaesthetic effect between optical antipodes have been reported previously (SCHAUMANN 1953, MOTOVILOV 1961; ÅKERMAN, PERSSON & TEGNER 1967) For some molecules these differences were related to differences in rate of metabolism or absorption of the isomers

In the present study enantiomers of a number of spirosuccinimides were also found to differ in their effect *in vitro* on isolated preparations of intact and desheathed nerves as well as on single fibres This suggests that the antagonistic effect of these compounds to impulse propagation may involve an interaction with specific structures in the excitable membranes

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Acta pharmacol et toxicol 1967, 25 suppl 4 55

From the Department of Pharmacology, Turku University Turku Finland

Bioassay of Prolonged Corticotropin Preparations, with the International Working Standard on Living Guinea Pigs

By

Pekkarinen, Aimo

Prolonged corticotropin preparations (gelatine, carboxymethylcellulose (CMC), polyphloretinphosphate (PFF) and Zn-phosphate) were standardized by using the prolonged preparations of the international working standards, as the reference standards (Mill Hill, London) in 4- and 6-point assays on living guinea pigs after preptreatment with pentobarbitone (Abbot 10 mg/kg) sublingually, methadone (Leiras 4 mg/kg) i p , and chlorpromazine (May & Baker 5 mg/kg) i p 2 hours before blood sampling For standardization male guinea pigs only are recommended Corticotropin was usually injected in the thigh of male guinea pigs 2 hours before blood sampling and in the case of carboxymethylcellulose, 3 hours before blood sampling The reference standard was usually injected in amounts of 0 033, 0 1 and 0 3 I U /100 g i m Blood was collected from the shoulder vein into a heparinized test tube and the 17 OHCS determined fluorimetrically In 6 successive standardizations, the mean potency of corticotropin in 15% gelatine (Lääke Oy) after dilution to 1 5‰ was in a 6-point assay 103 2 I U /mg, and in a 4-point assay 102 2 I U /mg Corresponding corticotropin powder has the potency 87 6 I U /mg

Ciba synthetic 1,24-corticotrophin-peptide in gela[illegible] [illegible] and 4-point assay in 6 and 3 series respectively

In 3 successive standardizations the mean potency of Ferring corticotropin peptide (No 40124) in 1% PFF (Leo) was between 143 9–134 6 I U mg in a 4-point assay, and 125 4–138 0 I U /mg in two series in a 6-point assay and as water soluble preparation in our bioassays 147 and 136 I U /mg in 4 series The mean potency of 1% carboxymethyl-

Acta pharmacol et toxicol 1967 25 suppl 4 54

From the Department of Pharmacology and Toxicology Royal Veterinary and *Agricultural College Copenhagen*

Effects of DDT on Reproduction in Hens

By

Marjun Weihe

Groups of 20 hens and two cocks were fed 0, 20, 200, and 1000 ppm DDT in their total diet for ten weeks

20 ppm did not influence reproduction as assessed by egg production, hatchability or survival of the chickens 30 ppm DDT and 10 ppm DDE were found in the yolk In the brains of chickens the concentration of *DDT reached 1 1 ppm*

200 ppm slightly reduced the survival of the chickens The concentrations of DDT and DDE in the yolk were at max 300 and 80 ppm resp, and in the brains of the chickens 13 ppm DDT

1000 ppm resulted in intoxication of the hens, reduced egg production and hatchability Tremor were observed in all chickens, and the mortality was 80–95 per cent in the first week The values of DDT and DDE were *1200 and 300 ppm in the yolk,* while in the chickens brains 40–130 ppm DDT were found

A concentration of 200 ppm DDT in the egg yolk seems to be the critical level for maintenance of normal reproduction in hens

Acta pharmacol et toxicol 1967, 25 suppl 4, 55

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cellulose corticotropin (Ferring No 129 P) was 145 3–153 6 I U /mg in two series of a 4-point assay and 152 8–167 8 I U /mg in two series of a 6 point assay, *and as water-soluble corticotropin on our earlier bioassay 140 9* I U /mg and 143 4 I U /mg respectively

Cortrophin Z (Organon 120 I U per ampoule) had a mean activity of 119 8 and 118 3 I U /ampoule in 6- and 4-point assays Cortrophin Z was dissolved with EDTA- and sodium citrate solution before bioassay

The mean index of precision in a 4-point assay is 0 19 and in a 6 point assay 0 18, and the mean confidence limits 82 2–121 7 per cent and 85 6–116 8 per cent respectively In 3 series of 11, 4-point bioassays and in 4 series of 11, 6-point bioassays statistical treatment showed an absence of either adequate linearity, parallelism, or curvature, which series showed a higher deviation of potency in bioassay

In 4 other successive standardizations, the combined mean potency of Organon Cortrophin Z was 112 9 I U /ampoule in a 4 point assay (ranges 107 9–117 1 I U) and 106 7 I U /ampoule in a 6 point assay (ranges 98 8–113 3 I U) and the mean index of precision was 0 15 and 0 14 respectively In one of these series in a 6 point assay statistical treatment showed the curvature

Acta pharmacol et toxicol 1967, 25 suppl 4, 57

From the Research Division, Pharmacia AB Uppsala, Sweden

Viewpoints on the Digital Computer Treatment of Biological Experimental Data

By

Dag E. S. Campbell

Calculation of data obtained in 10 different, recurring pharmacological and biochemical experiments has been performed for two years on the university computer CDC 3600 For each type of repeated experiments we developed a protocol scheme to collect the raw data items and a special Fortranprogram Usually this executed 250 items (minimum 35) and consisted of 270 statements representing an investment of 2700 Sw. kr Repeated execution with these programmes showed a median calculating time by hand and by computer of 14000 and 12 sec respectively. Computer time is charged at 1300 Sw kr /h, i e 100 times that of the assistant's salary Advantages of computer calculations are one tenth of manual cost, absolute reliability, clean output and ease of reversions A minimal set of data for one profitable computer performance is ½–1 hour of manual calculations Technicians directed independently computer calculations of their own raw data

Present manual calculations of experimental data in the various research disciplines at Pharmacia were investigated in order to forecast the possible future use of the computer Each of 134 experimentally active workers collected daily 34 items of raw data and performed calculations during 11 per cent of their time The same figures in toxicology were 85, 23%, pharmaco[illegible] armaceutics 45, 14%, control-an[illegible] To summarize, daily calculatio[illegible] ıbstituted by 7 computer min Of the raw data 70% originated from 30 instruments Connecting these to an automatic data collecting system might substitute for 3 assistants only

Acta pharmacol et toxicol 1967, 25 suppl 4 58

From the Department of Pharmacology, University of Copenhagen and Medical Department, Sankt Lukas Stiftelsens Hospital, Copenhagen, Denmark

The Effect of Glycopyrrolate on Mucus in Gastric Juice Determined by a Potentiometric Procedure

By

Marie Kristensen

The mucus in gastric juice was determined as non-volatile buffer substance by a modified potentiometric procedure For titrating the gastric juice, the investigator used a Radiometer titrator (Type TTT 1 h) and titrigraph (Type SBR 2c) For each titration 1875 µl gastric juice was used, and to ensure complete saturation of the buffer substances in the gastric juice with hydrogen ions 225 µl 1 N hydrochloric acid was added to the samples before titration The automatic titration was carried out with 2 N sodium hydroxide A difference in the course of the titration curves for gastric juice and a blank sample, approximately identical with gastric juice except for the abscence of buffer substances, indicates titration of the buffer substances in the gastric juice The quantity of sodium hydroxide used for the latter was a useful measure of the concentration of mucus determined as non-volatile buffer substances

Gastric juice was continuously aspirated for 5 hours from 7 patients with peptic ulcer The effect of glycopyrrolate was assessed on the basis of the secretion under basal conditions After one hour's recording of the basal secretion, 6 of the patients received 0 25 mg glycopyrrolate by intramuscularly injection In this way each patient served as his own control

In one patient who received no glycopyrrolate at all, the variations in basal secretions during 5 hours were equal to the variations found in the basal hour of the 6 patients before glycopyrrolate administration

In 5 of the 6 patients, glycopyrrolate increased the concentration of mucus in the gastric juice In all 6 patients it reduced the output of mucus The greatest reduction was seen in the second hour after injection of glycopyrrolate The reduction was then 75% for 3 patients with duodenal ulcer and 65% for 3 patients with gastric ulcer

Acta pharmacol et tox col 1967 25 suppl 4 59

From the St. Hans Hosp tal Dept E. Roskilde Denmark

Role of the Corpus Striatum in Typical Behavioral Effects in Rats produced by both Amphetamine and Neuroleptic Drugs

By

R L Fog

In rats intracerebral micro injections of quaternary chlorpromazine and related drugs in the corpus striatum give rise to behavioral effects which are highly characteristic for neuroleptic drugs (JANSSEN *et al* 1965) antagonism of amphetamine induced stereotyped behavior and develop ment of catalepsy Control injections into the hippocampus and septum have no effect (FOG *et al* 1966)

Intracerebral micro injections into the corpus striatum of dopamine or of dopamine plus an anticholinergic agent can restore amphetamine induced stereotyped behavior which has been inhibited by a methyl tyrosine (a drug which inhibits the formation of dopamine from tyrosine) Control injections of placebo and injections in the cortex had no effect (FOG *et al* 1967)

Dopamine in the brain is highly concentrated in the corpus striatum Destruction of the corpus striatum through suction and electrocoagulation inhibits the development of stereotyped behavior after amphetamine (FOG *et al* 1966)

Dopaminergic mechanisms in the corpus striatum thus seem to play an important role in the action of amphetamine and of neuroleptic drugs

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Acta pharmacol et toxicol 1967, 25 suppl 4, 61

From the St. Hans Hospital, Dept E. Roskilde, Denmark

Production of a Stereotyped Behaviour in Rats by Dopamine in the Absence of Noradrenaline

By

J. Scheel-Krüger and A. Randrup

RANDRUP, MUNKVAD & UDSEN (1963) and RANDRUP & MUNKVAD (1967) have described the stereotyped behaviour induced in many species of animals by amphetamine The same form of a stereotype behaviour can be produced in rats after injection of l-DOPA, the physiological precursor of dopamine and noradrenaline (RANDRUP & MUNKVAD 1966)

In this paper the various forms of experiments are presented, which have been done to obtain a differentiation between the behavioural effects induced by dopamine and noradrenaline

Biochemical analyses have been made of these brain amines and their 3-o-methylated metabolites, 3-methoxytyramine and normetanephrine (SCHEEL-KRUGER & RANDRUP 1967)

Analyses were made at various times after l-DOPA, of the brain amines from rats pretreated with either a monoamine inhibitor (MAOI) pargyline or of rats first depleted of catecholamines by reserpine and then treated with nialamide (a MAOI) + l-Dopa

The best differentiation between the behavioural effects of noradrenaline and dopamine was however obtained, when diethyldithiocarbamate DDC was added to the treatment DDC blocks the biosynthesis of noradrenaline from injected DOPA but not that of dopamine (GOLDSTEIN 1966)

These analyses together with other results show that a continuous stereotype behaviour induced after injection of l-DOPA to rats is dependent on a high level of dopamine

Furthermore it is shown that this behaviour is independent of the levels and turnover of noradrenaline

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Acta pharmacol et toxicol 1967, 25 suppl 4, 60

From the Biological Department, National Pharmaceutical Laboratory, Stockholm and Department of Pharmacology, Kungl Farmaceutiska Institutet, Stockholm

Studies on Electrically Induced-Seizures and Their Antagonism by Anticonvulsants During Neonatal Development in the Mouse

By

H. Ferngren and L. Paalzow

A new method for electroshock seizures in animals which was introduced by Paalzow (Paalzow 1966) has been modified for mice during the neonatal period Suitable electrical parameters for seizures (extension of forelimbs) were determined and found to be constant from birth to 9 days of age (Frequency 100 periods per second, pulse width 4 milliseconds, duration of chock 800 milliseconds) As parameter for the anticonvulsant effect, the reduction of the duration of the seizure was chosen 1-day-old, 3-day-old, 5-day-old and 9-day-old mice were investigated The anticonvulsant effect of various subcutaneous doses of phenobarbitone, diphenylhydantoin, ethosuximide, trimethadione and acetazolamide were compared with the effect of control solutions and followed up during 24 hours All drugs except ethosuximide showed the most marked effect in 5-day-old mice Ethosuximide had the best effect in 9-day-old animals Differences in the duration of the effect were found between drugs for the same age, and between ages for the same drug Diphenylhydantoin seemed to have the longest duration of action, over 24 hours in all age groups except in 5-day-old animals

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Acta pharmacol et toxicol 1967, 25 suppl 4, 63

From the Department of Pharmacology, H Lundbeck & Co, A/S, Copenhagen

Potentiation of Apomorphine Effect (Compulsive Gnawing Behaviour) in Mice

By

V. Pedersen

In rodents, apomorphine has been reported to induce a compulsive gnawing behaviour and an increased locomotor activity In mice apomorphine has only a weak effect Thus, a dose as high as 20 mg/kg s c. does not induce a typical gnaw-compulsion When pretreated with a compound, which potentiates the effect of apomorphine, the mice exhibit an intense gnaw-compulsion syndrome A modification of the apomorphine test of THER & SCHRAMM (1962) is described and the results of an investigation of a series of anticholinergics, thymoleptics and neuroleptics will be presented The highest gnawing intensities were obtained with the thymoleptic drugs

The apomorphine potentiating effect of a number of thymoleptics and centrally acting anticholinergics was studied in mice pretreated with physostigmine and flupenthixol Physostigmine caused a significant inhibition of the effect of the anticholinergics, while the effect of the thymoleptics was only slightly reduced After flupenthixol pretreatment the effect of the anticholinergics was inhibited to a lesser degree than that of the thymoleptics The results obtained seem to indicate that an anticholinergic effect is not the only important factor concerned in the apomorphine potentiating effect of a compound

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Acta pharmacol et toxicol 1967, 25 suppl 4 62

From the Sct Hans Hospital dept E, Roskilde Denmark

Brain Dopamine and Amphetamine-induced Stereotyped Behaviour

By

A. Randrup and I. Munkvad

Amphetamine causes a motor excitation which at moderate doses (about 10 mg/kg d-amphetamine-sulphate) assumes a stereotyped persisting form In rats this stereotypy consists of continuous sniffing, licking or biting

Both excitation and stereotypy can be prevented by α-methyl-tyrosine which inhibit the organism's synthesis of DOPA and accordingly that of the catecholamines, dopamine and noradrenaline If the rats are treated with the dopamine-β-oxidase inhibitor diethyldithiocarbamate (DDC), which inhibits only the synthesis of noradrenaline, the motor activity (such as locomotion) is reduced, but the stereotyped sniffing-licking-biting is just as constant and without interruptions, though the movements may be more slow Adrenergic blocking agents have similar effects This indicates that amphetamine interacts with the brain dopamine causing stereotypy, while noradrenaline seems to be of importance for other forms of activity, i a locomotion

Reserpine depletes both dopamine and noradrenaline from the brain but does not inhibit amphetamine-stereotypy However, ANDÉN, ROOS and WERDINIUS have found that the synthesis of dopamine still continues in the brain Our experiments have now shown that amphetamine affects this turnover and causes a sharp increase of the O-methylated metabolite of dopamine, while the corresponding metabolite of noradrenaline is not influenced It is thus possible that amphetamine, too when given after reserpine, acts conjointly with brain dopamine (RANDRUP *et al*)

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Acta pharmacol et toxicol 1967, 25 suppl 4, 65

From the Pharmacological Laboratory A/S Alfred Benzon,
Copenhagen Denmark

Demonstration of a Prolonged Analgesic Action in Animal Experiments

By

Sune Jespersen

The analgesic activity of a sustained-release preparation of dextropropoxipheni chloridum NFN (ABALGIN RETARD ®) as compared to that of dextropropoxiphene chloride solutions, was investigated in animal

A method is described for the oral administration of the small pellets forming the sustained release preparation

The analgesic effect was determined partly by means of the phenylquinone test in mice, using various intervals when administering the test substances before phenylquinone, and partly by means of a mechanical pressure test in rats (the RANDALL & SELITTO test), in which the pain threshold of each animal is determined several times

The results obtained from the experiments with both mice and rats indicate an essentially more prolonged and regular effect of the sustained-release preparation than of dextropoxiphene given as a simple solution

REFERENCE

Randall & Selitto A method for measurement of analgesic activity on inflamed tissue *Arch Int Pharmacodyn* 1957 111, 409

Acta pharmacol et toxicol 1967 25 suppl 4 64

From the Department of Pharmacology University of Uppsala Sweden

Synergism Between Hexobarbitone and Amphetamine on the EEG Burst-Suppression Threshold

By

E Widerlov and G Wahlstrom

The amount of racemic hexobarbitone sodium (evipan ®) needed to obtain a burst suppression of 1 sec or more in the EEG during a continuous intravenous infusion (0 25 mg/kg/sec) was used as the threshold (WAHLSTRÖM 1966a) The experimental animals were male Sprague Dawley rats (weight around 400 g) Shortly after the EEG criterion had appeared, the infusion was terminated The ensuing sleeping times were measured on automatic beds, which recorded the time at which the righting reflex was absent (WAHLSTRÖM 1966b)

d l amphetamine (15 mg/kg) was given intraperitoneally 1, 3 and 6 hours before the threshold determination A decrease in threshold was found with a maximum after 1 hour This decrease was 16 9% (s e m = 2 9% n = 18) calculated on the average of three preexperimental values After 3 hours the decrease in threshold was 14 3% (s e m = 3 4% n = 10) and after 6 hours it was 12 9% (s e m = 2 6% n = 7)

The sleeping times after the threshold doses were reduced The decrease was 33 1% (s e m = 8 1% n = 13) after 1 hour and 17 0% (s e m = 9 8% n = 8) after 3 hours

Amphetamine thus caused a clear reduction in the amount of hexobarbitone needed to obtain the EEG criterion No definite explanation for this observation can be given at present It is probably not related to an increase in cerebral blood flow (which would increase the rate at which barbiturate reaches the brain) as 5% CO_2 had only a slight effect on the threshold (WAHLSTRÖM 1966a)

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Acta pharmacol et toxicol 1967, 25 suppl 4 65

From the Pharmacological Laboratory A/S Alfred Benzon
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Acta pharmacol et toxicol 1967, 25 suppl 4, 66

From the Leo Pharmaceutical Products, Ballerup, Denmark

Experimental Study on Combined Treatment with Phenobarbitone and Diphenylhydantoin

By

H.-H. Frey, Elsemarie Kampmann and Chr. Kærgaard Nielsen

The knowledge that phenobarbitone by induction of liver microsomal enzymes is able to accelerate the metabolic breakdown of diphenylhydantoin (Cucinell *et al* 1963) has cast some doubt on the value of the combined use of both drugs in the treatment of epilepsy Hence, the interaction of both drugs was followed in dogs The animals received daily doses of 3 mg/kg phenobarbitone and/or 10 mg/kg diphenylhydantoin orally over a period of 30 days, and the half-lives of both drugs were determined before, as well as during and after treatment In addition to the well-known fact that phenobarbitone is able to accelerate its own metabolism, a shortening of the half-life for diphenylhydantoin was shown in some of the experiments, while other experiments especially those showing a short half-life at the start, even showed a prolongation or no change at all The mutual interactions of both drugs thus seem to be unpredictable and a laboratory control of the serum concentrations is necessary if toxic actions and insufficient control of the disease are to be avoided It is stressed that the patient may reach extreme rates of drug metabolism as a consequence of other drug therapy, as well as of occupational exposure to, for example, pesticides or chemicals

REFERENCE

Cucinell S A, R Koster A H Conney & J J Burns Stimulatory effect of phenobarbital on the metabolism of diphenylhydantoin *J Pharmacol exp Ther* 1963 141 157 160

Acta pharmacol et toxicol 1967, 25 suppl 4, 67

From the Research Division Pharmacia AB Uppsala, Sweden

Pharmacological Effects of a New Antidepressant Drug

By

W. Richter

Potential thymoleptic activity was detected in several members of a series of tricyclic structures subjected to screening for anti-reserpine activity in mice The most active compound, 0-(2-Dimethylaminoethyl)-10,11-dihydro-5H-dibenzo [a, d] cyclohepten 5 one oxime hydrochloride (code name CD 37B), was further characterized and compared with established antidepressants, using a battery of tests in mice, rats, guinea-pigs, cats and dogs In the table below, some of the results are presented, showing that CD 37B exhibits a profile of effects qualitatively similar to but, quantitatively different from that of the reference drugs Like to imipramine, CD 37B inhibits the membrane pump mechanism for the uptake of catecholamines (B E Roos 1967, personal communication) CD 37B does not appreciably affect blood pressure and respiration in anesthetized rabbits and cats A chronic toxicity study in rats did not disclose any organic or functional changes In preliminary clinical trials, CD 37B has been found to have antidepressant effect

I = imipramine, A = amitriptyline, DMI = desmethylimipr , DMA = desmethylamitr P or A = *presence or absence of effect 0, +, ++, +++, ++++ = relative strength*

Effect or parameter measured	CD37B	I	A	DMI	DMA
Potentiation of barbiturate anaesthesia, mouse	+	+	+++		
Decrease of spontaneous motor activity, mouse	+	++	++		
Decrease of body temperature, mouse	+	++	+++		
Abolition of fighting behavior, mouse	+++	++	+++		
Abolition of amphetamine hypermotility, mouse	0	0	0	0	0
Elevation of body temp in reserpinized mice	++++	++	++	+++	++/+++
Antagonism of reserpine ptosis, mouse	+++	+	++	+/++	
Eff against prochlorperazine catalepsia, mouse	++++	+	++++		++++
Potent of isoprenaline hyperthermia, mouse	++++	+++	++		
Potent of noradrenaline pressor effect, cat	P	P	P		
Incr body temp in nialamid treat guinea pig	++/+++	++++	++	+	0
Abolition of tremorine tremor, mouse	+++	++	+++	+	++
Anti acetylcholine, isol ileum guinea pig	+++	+	++++		+
Mydriatic effect, mouse	++++	++	+++		
Antagonism of pilocarpine salivation mouse	++	+	++		
Anti histamine, isol ileum, guinea pig	+/++	++	+++		++++
Anti barium eff , isol ileum guinea pig	+++	+++	+++		++
Abol of leptazol convuls , extens comp mouse	+	0	++		
Elevation of leptazol seizure threshold mouse	0	0	0		
Analgesic effect tail flick mouse	+	++/++	+		
Local anesthetic effect rabbit cornea	+	+ +		+	

Acta pharmacol et toxicol 1967, 25 suppl 4 69

From the Research Division Pharmacia AS Copenhagen Vanløse

Pharmacological Investigation of 3-(β-dimethylaminoethyl)-2-thio-2,4-[1H, 3H]-quinazoline-dione, hydrochloride (QB 15B)

By

Jan Weis

In vitro experiments showed that QB 15B stimulates the isolated guinea pig ileum Among the antagonists tested, atropine was shown to be a specific inhibitor of contractions elicited by QB 15B, suggesting a cholinergic mechanism

In the acetic acid writhing test, QB 15B was highly active like morphine, chlordiazepoxide, or atropine It was, however, inactive in the tail flick test and is therefore not a "morphine type" analgesic.

The lack of activity in five different experimental inflammation models and the absence of an antipyretic effect on yeast induced fever in rats, clearly excludes QB 15B from the class of "antiphlogistic" analgesics

The structural similarities of quinazolines and 1,4-benzodiazepines, of which the latter have been shown to possess narcotic antagonistic properties (CARRABATEAS & HARRIS 1966) prompted the further study of QB 15B s activity against morphine in the tail flick test in mice Appreciable antagonism to morphine was observed with doses of 10 mg/k - while Chlord

Attempts to characterize QB 15B in a few psychopharmacological tests are described No effect on the reserpine induced hypothermia similar to that exhibited by thymoleptics or MAO inhibitors was observed Pentylenetetrazol induced convulsions and barbiturate sleeping time, common tests for muscle relaxant tranquillizers and neuroleptics remained unaffected Amphetamine hyperthermia in mice was, however, potentiated by both QB 15B and chlordiazepoxide

The pharmacological spectrum of QB 15B

REFERENCE

Carrabateas, P M & L S Harris Analgesic antagonists 3 4-substituted 1 acyl 2,3 4 5-tetrahydro-1H 1 4-benzodiazepines. *J Med Chem* 1966 9, 6–10

I = imipramine, *A* = amitriptyline, DMI = desmethylimipr , DMA = desmethylamitr P or A = presence or absence of effect 0, +, ++, +++, ++++ = relative strength

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Elevation of body temp in reserpinized *mice*	++++	++	++	+++	++/+++
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Anti barium eff , isol ileum guinea pig	+++	+++	+++		++
Abol of leptazol convuls , extens comp , mouse	+	0	++		
Elevation of leptazol seizure threshold *mouse*	0	0	0		
Analgesic effect tail flick mouse	+	++/++	++		
Local anesthetic effect, rabbit cornea	+	+++	++	++	

Acta pharmacol et toxicol 1967 25 suppl 4 71

From the Institute of Psychology and Research Division Pharmacia AB
Uppsala Sweden

Behavioural Pharmacology in Pigeons and Rats of a New Tricyclic Antidepressant

By

Ingmar Dureman and Bengt Henriksson

Various experiments were performed on trained pigeons and rats Of these, some representative examples are described

1) Three pigeons had previous training on a FI 5 TO 2 schedule for 6 months During the experimental period these pigeons had four control days and one day on drug per week On food reinforced pecking, CD 37B at a dose of 3–5 mg/kg intramuscularly produced an initial over all decrease of pecking activity lasting for about 25 minutes, followed by an accelerated pecking towards the end of the 50 minutes experimental period The same effect was also seen after the same dose of imipramine With CD 37B, as after imipramine, there was also a marked attenuation of the typical pausing following each reinforcement at all dose levels With doses of 7 and 10 mg/kg the neuroleptic component of the over all decrease of pecking rate was more marked and more prolonged than that after comparable doses of imipramine This suggests a stronger neuroleptic effect with increasing doses of CD 37B as compared with imipramine

2) Two groups of five albino rats were trained to escape a strong noise (group 1) or avoid the same aversive stimulus during an 8 second forewarning period with a weak noise (group 2) by opening a switch door by pressing a lever in the floor of an acoustic stress chamber

After one month of training the average non-drug control value for escape latency for group 1 was 1 24 seconds After the same period the average avoidance latency for group 2 was 1 39 seconds

20 mg/kg CD 37B given subcutaneously 60 minutes before the first drug trial, resulted in a considerable increase in avoidance latency averaging 160%, whereas the escape latency remained stable at the control level In a subsequent study a dose level of 5–7 mg/kg of chlorpromazine was found to yield the same relative increase in avoidance latency without any effects on the escape latency

3*

Acta pharmacol et toxicol 1967, 25 suppl 4 70

From the Research Laboratories of *H Lundbeck & Co*, *A/S*, Ottiliavej 7, Copenhagen, Valby

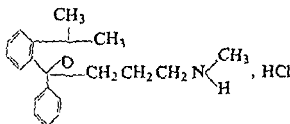
Lu 3-olo (3,3 dimethyl-1-phenyl-1-3 methylaminopropylphthalane): Pharmacological profile

By

I. Møller Nielsen

In a previous publication (PETERSEN *et al* 1966) *the results of a pharmacological* screening of a series of bicyclic compounds with regard to "thymoleptic" properties were reported

A particularly interesting substance, Lu 3-olo with the following structure,



proved in some respects to be more potent than the most active tricyclic thymoleptics, protriptyline and desipramine

Lu 3-olo was found to be a very potent potentiator of catecholamines, presumably due to inhibition of the amine pump, while blockade of adrenergic receptors could not be demonstrated even with high doses Correspondingly Lu 3-olo showed strong antagonism against reserpine-induced ptosis, with no tendency to reversal with high doses

In contrast to the tricyclic thymoleptics, Lu 3-olo did not potentiate the effect of apomorphine in mice and showed extremely weak peripheral anticholinergic activity

In pithed rats Lu 3-olo potentiated the pressor response to 5-HT, while tricyclic thymoleptics as a rule are 5-HT-antagonists

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Acta pharmacol et toxicol 1967, 25 suppl 4 73

From the Research Institute of National Defence Sundbyberg 4 Sweden

Evaluation of the Central Effects of Atropine in the Dog

By

Lennart Albanus, Sten-Magnus Aquilonius, Anders Sundwall and Birger Winbladh

Following subcutaneous injection of atropine in doses higher than 0 5 mg/kg, typical effects on behaviour occur in addition to the well known peripheral symptoms These central effects mainly consist of ataxia and decreased environmental awareness The same symptoms have also been elicited by intraventricular injections of atropine (EDERY 1962) It has been suggested that these effects might be caused by the penetration of atropine from the ventricles into the adjacent brain structures (EDERY 1962, FELDBERG 1963) The present study has been undertaken to elucidate the relationship between the pharmacological effects and the critical plasma levels of the drug after different routes of administration

Atropine was injected into the lateral ventricles in concious dogs through permanently implanted cannulas It was found that the central effects were not obtained with smaller doses than those given subcutaneously, i e 0 5 mg/kg Injection of T-labelled atropine showed that absorption into the blood was as fast as after subcutaneous injection Furthermore the typical central effects were obtained at the same plasma level (0 1 μg/ml) with both routes of administration However, a marked hyperthermia occurred which was not obtained even after a hundredfold dose given subcutaneously

The results suggest that the typical central symptoms are elicited when a critical plasma level is reached The hyperthermia, on the other hand, is probably caused by a direct spread from the cerebrospinal fluid

REFERENCES

Edery H J pharmacol chemotherapy 1962, 18 19

Feldberg, W A pharmacological approach to the brain Arnold Publ Ltd London 1963

Acta pharmacol et toxicol 1967, 25 suppl 4 72

From the Department of Pharmacology University of Uppsala Sweden

Changes Induced in the Self-selected Circadian Rhythm of the Canary by Thriiodothyronine and ^{131}I

By

Goran Wahlstrom

The self-selected circadian rhythm has been recorded in canaries kept singly in lightproof cages The light inside the cage (an ordinary lamp) is regulated by a perch When the bird hops onto this perch the light goes out and when it leaves the perch the light is turned on The canaries can thus choose between light and darkness There is usually only one activity period (light) and one rest period (darkness) in the circadian period The circadian period is counted from waking up of the bird (start of the activity period)

Thriiodothyronine (6–10 mg/kg orally) administered as a single dose to normal birds late during activity ($n = 37$) caused an increase in activity and a decrease in rest with a maximal response in the first period after the administration The circadian period was also increased with a maximum in the first period Similar treatments early during activity ($n = 7$) gave no clear cut results

Several treatments in the same bird with ^{131}I (10–20 µg/dose) gradually caused a decrease in activity, an increase in rest and a decrease in the circadian period Thriiodothyronine (6–10 mg/kg orally) given as a single dose late in the activity to birds previously treated with ^{131}I ($n = 5$) caused an increase in the circadian period similar to that in normal birds though more marked The increase could be traced over 9–10 periods after administration During the first three periods, changes in activity and rest were opposite to those obtained in normal birds but after this the changes were similar

The present results gave a clear indication that the thyroid has an effect on the length of the self-selected circadian period Curiously enough the positive effect was a longer circadian period

Acta pharmacol et toxicol 1967, 25 suppl 4 73

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Acta pharmacol et toxicol 1967, 25 suppl 4 74

From the Department of Pharmacology, University of Copenhagen Denmark

Cholinesterase and Protein Changes in Sympathetic Ganglia after Guanethidine*)

By

J. Jensen-Holm

Guanethidine sulphate (10 mg/ml in saline) injected intraperitoneally into albino rats (20 mg/kg daily for 6–14 days) caused an increase in the size and the content of protein in the superior cervical ganglia of 20 to 80%, when the ganglia were removed three days after the last injection Specific and non-specific cholinesterase activity was reduced by 50–60% per ganglion, and by 70–80% per mg of protein Similar changes were found in the stellate ganglion 16 days after discontinuation of treatment, the activity of cholinesterases had returned to about 70% of the corresponding control values

These changes are similar to the effect of axotomy in rats (among others, see Brown 1958) but not in cats (Gromadzki & Koelle 1965)

Saline injection alone (2 ml/kg daily i p for 6–14 days) caused a slight reduction in the amount of protein and an increase in the activity of cholinesterases (per mg of protein or per superior cervical ganglion)

The effects of guanethidine and of saline described will be further investigated

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Brown Lucy M Cholinesterase in the superior cervical ganglion of the rat after preganglionic denervation and axotomy *J Physiol (Lond)* 1958 142 7P–8P

Gromadzki, Cornelia G & G B Koelle The effect of axotomy on the acetylcholinesterase of the superior cervical ganglion of the cat *Biochem Pharmacol* 1965 14 1745 1754

*) A preliminary report was presented at The British Pharmacological Society Meeting April 1967 (Mill Hill) with Eleanor Zaimis (University of London)

Acta pharmacol et toxicol 1967, 25 suppl 4 75

From the Research Institute of National Defence Sundbyberg 4 Sweden

Incorporation of Exogenous Acetylcholine, Choline and Atropine in Mouse Brain Cortex Slices

By

J. Schuberth and A. Sundwall

It has recently been shown that choline (Ch) and acetylcholine (AcCh) are taken up against a concentration gradient when brain cortex slices are incubated in a suitable medium (SCHUBERTH *et al* 1966, POLAK & MEEUWS 1966) Using tritium labelled atropine we have shown that this drug is also accumulated in cortex slices against a concentration gradient In the present communication we have compared the uptake of Ch, AcCh and atropine with regard to inhibition by drugs and also subcellular distribution

Hemicholinium (HC-3), eserine, atropine, oxotremorine and morphine were found to be competitive inhibitors of the uptake of AcCh with K_i values 0 5 10^{-5}, 0 7 10^{-5}, 1 6 10^{-5}, 2 3 10^{-5} and 3 6 10^{-5} M Of these drugs only HC-3 was found to inhibit Ch uptake (K_i 0.6 10^{-4} M) Thus HC-3 is a ten times stronger inhibitor of AcCh [illegible]

[illegible]

Atropine uptake was not affected by any of the drugs tested

Studies on the subcellular localization of the drugs taken up by the slices revealed that the main part of the radioactivity was concentrated in the nerve-ending fraction following incubation with Ch or AcCh In contrast no specific subcellular localization of [illegible] after [illegible] of rac[illegible] ing in [illegible]

The results thus clearly show that Ch and AcCh uptake differ from that of atropine both with regard to the susceptibility to drugs and to subcellular distribution

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J Schuberth A Sundwall B Sörbo & J O Lindell *J Neurochem* 1966 13 347
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Acta pharmacol. et toxicol 1967, 25 suppl 4, 77

From the Research Institute of National Defence, Sundbyberg Sweden

Metabolic Disposition of Methylatropine

By

L. Albanus, A. Sundwall and B Vangbo

The metabolism of methylatropine has apparently not been reported in the literature

In the mouse tritium labelled methylatropine (5 mg/kg was injected intravenously or given by stomach tube Urine was chromatographed in different solvent systems or separated by high voltage electrophoreses

In addition to unchanged methylatropine (about 40% after 2 hr) two major metabolites were found (14 and 35% after 2 hr) in the urine None of these was split by β-glucuronidase Several organs were assayed for radioactivity The concentration of radioactivity in the brain was only about 7% of that in blood, while the concentrations in heart muscle, salivary glands, lung, stomach and bile were considerably higher than in blood Following oral administration maximum plasma concentrations were reached after 60 min and the concentration was about half of that found after intravenous injection Practically all radioactivity in the urine was in the form of metabolites

In the dog 0 1 mg/kg tritium labelled methylatropine iodide subcutaneously produced a marked tachycardia within 5 minutes Following oral administration of the same dose, no effect on heart rate was observed during a 6 hr observation period

Analysis of the plasma levels showed a peak concentration of radioactivity after 20 minutes following subcutaneous injection and after 120 minutes following oral administration There was a ten fold difference between the peak concentrations After 6 hr 60% of the radioactivity was excreted in the urine following subcutaneous injection, while only 20% was excreted during the same period following oral administration During the next 18 hr an additional 10% was excreted following subcutaneous injection as against only 2% after oral administration

Acta pharmacol et toxicol 1967, 25 suppl 4 76

From the Department of Pharmacology and Toxicology, Royal Veterinary and Agricultural College, Copenhagen, Denmark

Atropine Substitutes alone and in Combination *with TMB-4* used as Protection against Paraoxone Intoxication in Mice

By

Ole Karlog

After prophylactic treatment with atropine, scopolamine, methyl-scopolamine, benztropine, N-methylpiperidinphenylcyclopentancarboxylate, benactyzine, caramiphene, 3-quinuclidinylphenylcyclopentancarboxylate, 3-quinuclidinyldiphenylacetate and 3 quinuclidinylbenzilate, and also after each of these compounds in combination with TMB-4 (N,N'-trimethylene-bis (pyridine-4-aldoxime chloride)) the LD50 for paraoxone (0,0-diethyl-0 p-nitrophenylphosphate) was estimated in female mice

Atropine and atropine substitutes were injected intraperitoneally (3 µmol base/kg bdw) 10 min before the subcutaneous injection of paraoxone

TMB-4 (20 mg/kg bdw) was also injected intraperitoneally 10 min before paraoxone

Atropine alone protected against 0 89 mg paraoxone/kg bdw (equivalent to 1 3 × LD50 for paraoxone) The protective effect of scopolamine and 3-quinuclidinylbenzilate was twice that of atropine, while the effect of the other compounds was equal to atropine

In combination with TMB-4, scopolamine, 3-quinuclidinylphenylcyclopentancarboxylate and 3-quinuclidinylbenzilate were 7, 8, and 16 times more effective than atropine in combination with TMB-4

6 6 mg atropine, 2 4 mg scopolamine, 1 8 mg benztropine and 0 18 mg 3-quinuclidinylbenzilate/kg in combination with TMB-4 were capable of protecting 50% of the mice against 175 mg paraoxone/kg bdw (equivalent to 250 times LD50 for paraoxone)

Acta pharmacol et toxicol 1967, 25 suppl 4 79

Department of Pharmacology and Department of Histology, Karolinska Institutet Stockholm Sweden

Identification of Sympathetic Cholinergic Nerve Terminals in Arterioles of Skeletal Muscle

By

Per Bolme and Kjell Fuxe

There is physiological and pharmacological evidence that synaptic transmission at the postganglionic terminals of the sympathetic vasodilator nerves is mediated by *acetylcholine* These nerves are considered to innervate the arterioles of skeletal muscle. By means of the thiocholine method as modified by HOLMSTEDT (1957), skeletal muscle of the dog and of some other species was stained for the presence of acethylcholinesterase (AcChE) In the dog, AcChE-containing structures with the appearance of nerve terminals were found surrounding arterioles 30–100 μ in diameter After unilateral chronic lumbar sympathectomy, these "nerve terminals" disappeared in the muscles of the hind limb on the operated side Muscles were also stained for the presence of noradrenaline, the adrenergic transmitter, by the histochemical fluorescence method of FALCK & HILLARP The experiments suggest that the cholinergic vasodilator nerves as well as the adrenergic vasoconstrictor nerves are localized in the same layer of the arterioles i e in the adventitia surrounding the outer sheeth of the *muscularis*

Moreover preliminary experiments indicate, that cholinergic vasodilator nerve terminals are present in skeletal muscles of the cat and of the sheep but not of the badger These results agree with the physiological findings on this subject (BOLME, NOVOTNY, UVNÄS & WRIGHT, unpublished)

REFERENCE

Holmstedt B [illegible]

Acta pharmacol et toxicol 1967, 25 suppl 4, 78

From the Department of Pharmacology, University of Umeå, Sweden

The Occurrence of Soluble Acetylcholineesterases in Mammalian Brain

By

G. Hollunger and B. Niklasson

About 12% of the acetylcholine hydrolysing activity in tissue homogenates of the caudate nucleus from calf brain is found in the supernatant 2 hours after centrifugation at 100,000 g Immediate gel filtration of the supernatant on a Sephadex G-200 column resolves the activity into four peaks, one of which is excluded, the other three appearing at elution volumes corresponding to molecular weights of about 510,000, 240,000 and 85,000, respectively The greater part of the activity is found in the low molecular weight enzyme peak The enzymes are true acetylcholineesterases as judged by their substrate and inhibitor specificities Gel filtration 24 hours after the preparation of the supernatant reveals a decrease of the activity in the low molecular peak and a corresponding increase in the other peaks This aggregation can be avoided by purifying the low molecular enzyme by an ammonium sulphate fractionation – From the particulate fraction of the homogenate, a substantial amount of the acetylcholineesterase activity can be released solely by resuspension and incubation of the particles for some hours It is thus possible that the soluble acetylcholineesterases demonstrated have been solubilized during the preparation and hence do not represent naturally occurring forms of the enzyme

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THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE CONCENTRATION OF PENTOBARBITAL AND BARBITAL IN THE LIVER AND BRAIN

An experimental study on guinea pigs

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EERO SOTANIEMI

MUNKSGAARD COPENHAGEN 1967

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SWENSSON, ÅKE and ULF ULFVARSON (From the Clinic of Occupational Medicine, Karolinska Sjukhuset, Stockholm 60, Sweden) *Distribution and Excretion of Various Mercury Compounds after Single Injections in Poultry* Acta pharmacol et toxicol 1968, 26 (3), 259-272 (6 tables, 2 fig, 15 ref)

Abstract

White leghorn cocks were injected i v with aqueous solutions of mercury (Hg^{203}) compounds in doses of approximately 1/5 LD50

Ten days after the injection of 500 µg of $Hg(NO_3)_2$ (I) the following values were found in blood 4 9, in liver 1416, in kidneys 1973, in muscles 18 2 ng/g Hg, after 6000 µg/kg of methyl mercuric hydroxide (II) in blood 4680, in liver 7220 in kidneys 8100, in muscles [illegible]

The excretion is hyperbolic and follows the general formula $\frac{1}{x} = k \times t + \frac{1}{x_0}$ (x_0 = initial content in µg/kg, x the concentration at the time t in days) The mean rates for k were for (I) $1\ 9 \times 10^{-4}$, for (II) $3\ 7 \times 10^{-6}$, for (III) $1\ 1 \times 10^{-4}$, and for (IV) $7\ 1 \times 10^{-5}$ $(µg/kg)^{-1}$ day^{-1}

SWENSSON ÅKE and ULF ULFVARSON (From the Clinic of Occupational Medicine, Karolinska Sjukhuset, Stockholm 60, Sweden) *Distribution and Excretion of Mercury Compounds in Rats over a Long Period after a Single Injection* Acta pharmacol et toxicol 1968, 26, (3), 273 283 (4 tables 4 fig, 5 ref)

Abstract

Female albino rats were injected i v with aqeuous solutions of mercury compounds (Hg^{203})

The total excretion of mercury in the body was found to follow the equation $\frac{1}{x} = k\ t + \frac{1}{x_0}$ (x_0 – initial concentration of Hg in µg/kg, x the concentration of Hg at time t in days) Phenylmercuric hydroxide (I) and mercuric nitrate (II) showed [illegible]

AHTEE LIISA and MATTI K PAASONEN (From the Department of Pharmacology Univers ty of Hels nki Finland) *Potent at on of 5 Hyd oxytryptam ne Release From Platelets by Desmethyla on of Chlorproma ne and Related Agents* Acta pharmacol et toxicol 1968 26 (3) 213 221 (2 tables 2 fig 19 ref)

Abstract

Platelets were obta ned from rabb t blood and ncubated at 37 for 30–180 m n Ad d t on of 10^{-4} M chlorpromaz ne (I) or im pramine (II) produced a 5HT release of 15–20% after 3 hours 10^{-4} M am triptyl ne released 30% Desmonomethyl chlorpromazine or des m pram ne 10^{-4} M caused a depletion of about 60% and nortriptyl ne of 70% The correspond ng pr mary am nes of (I) or (II) showed approximately the same depleting power as the secondary am nes The effect of a concentrat on of 3×10^{-5} was almost negl g ble and w th 3×10^{-4} M 70–95% 5HT had d sappeared The absorption into pla telets of (I) and its demethylated derivat ves from a 5×10^{-5} M solut on were 1 0 mg/ml of (I) 1 66 mg/ml of desmonomethyl chlorpromazine and 2 12 mg/ml of the pr mary am ne

The part t on coeffic ents between heptane or petroleum ether and a 0 1 M phosphate buffer (pH 7 4) increased markedly w th the number of methyl groups bound to the term nal N The concentrat on of (I) and its derivat ves after *in v tro* incubation was 10–20 t mes h gher in the platelets than in the erythrocytes

There was no rect l near correlat on between the haemolyt c effect and the capacity to l berate 5HT

OLESEN O VENDELIN (From the Central Laboratory the F ladelfia Colony D analund Denma k) *Dete m nat on og 5-(p-hydroxyphenyl) 5 phenylhydan o n (HPPH) in Ur ne by Th n Layer Chroma ography* Acta pharmacol et toxicol 1968 26 (3) 222 228 (2 tables 2 fig 16 ref)

Abs act

D phenylhydanto n adm n stered to man s ma nly excreted as 5 (p hydroxyphenyl) 5 phenylhydanto n (HPPH)

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m

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HCl taken as a measure of HPPH (E_{243} nm E_{241} nm) × 38 7 = mg/l HPPH

HPPH was d st ngu shed from phenobarb tal d phenylhydanto n and OH pheno barb tal

The recovery of added HPPH 11 350 mg l, was 91 4 3 2% Average error s = 2 8% The sens t v ty of the method was less than 10 mg/l urine

KIESSLING, K H (From the Institute of Zoophysiology, University of Uppsala, Uppsala, Sweden) *Effect of Ethanol on Rat Liver VI A Possible Correlation between α Gglycerophosphate Oxidase Activity and the Size of Mitochondria in Male and Female Rats fed Ethanol* Acta pharmacol et toxicol 1968, 26 (3), 245–252 (1 table, 1 fig, 23 ref)

Abstract

Two groups of rats were given ethanol 15% (v/v) in water or water with an isocaloric amount of sugar, respectively After 200 days, the animals were killed, and pieces of their livers examined in the electron microscope The mitochondria were isolated from other pieces for biochemical examination

The mean size of the male Wistar and Sprague-Dawley liver mitochondria was increased ($p < 0.001$), but not those of female Wistar rats The oxygen consumption of liver mitochondria, calculated per mg protein with pyruvate as substrate, was found to be significantly decreased (20%) in male Wistar rats and insignificantly uncreased in male Sprague-Dawley With succinate a decrease of 25% was found (male Wistars), there was no change in male Sprague Dawley With β hydroxybutyrate there was no change in Wistars and an increase of 8% in Sprague Dawley rats With α glycerophosphate there was an increase of 25% in Wistars and 70% in Sprague Dawley (both highly significant) No change was found in the metabolism of the Wistar female liver mitochondria

SCHOU, JENS (From the Department of Pharmacology, University of Copenhagen, Denmark) *A Comparison of the Potency of Adrenaline and Noradrenaline in Delaying Absorption from Muscles* Acta pharmacol et toxicol 1968, 26, (3), 253–258 (1 table, 3 fig, 5 ref)

Abstract

16 µl of 0 9 NaCl in water with tracer amounts of ^{14}C-sucrose or $^{3}H_2O$ and 0, 1, 2 5, 10 or 50 µg/ml of either adrenaline or noradrenaline was injected into the exposed extensor quadriceps femoris muscle of white rats

5 minutes after the injection appr 38% of sucrose remained in control experiments, while in the experiments with 1, 5, 10 and 50 µg/ml adrenaline or noradrenaline percentages of appr 36, 95, 95 and 96% were still present in the muscle respectively 15 minutes after sucrose injection 13%, 10%, 70%, 80%, and 85% of sucrose remained with 0, 1, 5, 10 and 50 µg ml adrenaline or noradrenaline, respectively

9 minutes after the injection of $^{3}H_2O$ with 0, 1, 5, 10 and 50 µg/ml adrenaline or noradrenaline appr 20, 20, 70, 80 and 80% of the injected radioactivity remained in the muscle

KIESSLING, K.-H. (From the Institute of Zoophysiology, University of Uppsala, Uppsala, Sweden) *Effect of Ethanol on Rat Liver VI A Possible Correlation between α-Gglycerophosphate Oxidase Activity and the Size of Mitochondria in Male and Female Rats fed Ethanol* Acta pharmacol et toxicol 1968, 26 (3), 245-252 (1 table, 1 fig, 23 ref)

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SECHER HANSEN, E (From the Department of Pharmacology, University of Copenhagen, Denmark) *Studies on Subcutaneous Absorption in Mice V Absorption of Water Injected into the Skin of Normal and Oestradiol Treated Animals* Acta pharmacol et toxicol 1968, 26 (3), 229–239 (1 table, 10 fig, 21 ref)

Abstract

Into one of two marked symmetrical areas on the back of albino male mice (Leo, Stritt) distilled water only, 0 9% NaCl or other substances in solution were injected The water was labelled with ^3H_2O The animals were killed after 5 or 15 min The symmetrical areas were isolated and anlysed Mice pretreated with 10 µg oestradiol monobenzoate (I) given 9 6 and 4 days before the experiment showed 60% higher residual water at the site of injection than untreated control mice, independently of whether distilled water, NaCl or sucrose solutions were injected Addition of hyaluronidase had no effect on the absorption in controls, but gave an almost complete absorption in mice treated with (I) After 15 min only about 25% of the ^3H_2O injected into (I) treated mice remained, 10% when hyaluronidase was added, and 5% in the untreated controls, with or without the addition of hyaluronidase, and irrespective of whether distilled water, saline or sucrose solution was injected Added ^{131}I labelled human serum albumin disappeared at the same rate as the injected volume decreased

SVEDMYR, NILS and ANDRAS BEVIZ (From the Metabolic Division, Department of Pharmacology, University of Göteborg Sweden) [illegible]

Abstract

Rats were given 0 1 mg thyroxine daily for 7 days, and this increased the O_2 consumption by 25–30%. The rats were killed and their diaphragms were cut in halves, one half was incubated in Krebs Henseleit [illegible] cubated with adrenaline [illegible] muscle were determined [illegible] dition of adrenaline (C [illegible] thyroxine treated rats (T), and the change after addition of adrenaline (T + A) adenosine triphosphate C 4 07 ± 0 27, C + A 0 56 ± 0 25, T 1 27 [illegible] 0 23, T + A +0 27 ± 0 31, adenosine diphosphate C 1 08 ± 0 07, C + A 0 15 [illegible] 0 15, T 1 13 ± 0 06, T + A +0 09 ± 0 11, adenosine monophosphate C 0 68 [illegible] 0 03 C + A +0 02 ± 0 04, T 0 65 ± 0 04, T + A +0 02 ± 0 05, creatine phosphate C 5 26 ± 0 52, C + A +1 15 ± 0 46, T 3 99 ± 0 37 and T + A +0 62 ± 0 52

The effect of adrenaline on the concentration of hexose phosphates was not altered thyroxine treatment

ACTA PHARMACOLOGICA ET TOXICOLOGICA
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ADDENDUM

The WHO names used in this study correspond to the following NFN names

WHO-name	*NFN name*
Barbital	Diemalum
Pentobarbital	Mebumalum
Phenobarbital	Phenemalum

FREY, H.-H., ELSEMARIE KAMPMANN and CHR. KÆRGAARD NIELSEN (From the Department of Pharmacology, Leo Pharmaceutical Products, Ballerup, Denmark) *Study on the Combined Treatment with Phenobarbital and Diphenylhydantoin.* Acta pharmacol. et toxicol. 1968, 26 (3), 284-292 (0 tables, 4 fig., 12 ref.)

Abstract

Three dogs (mongrels) were given 3 mg/kg/day of phenobarbital orally for 30 days. In one dog the half-life of phenobarbital decreased from 75 hours to 55 hours and then increased to 89 hours, in a second dog the half-life increased from 50 to 65 hours, then decreased within a week to 25 hours, and slowly rose to 44 hours within 2 months after discontinuation of phenobarbital. In the third dog, the half life fell from 62 to 36 hours and increased slowly to 55 hours after 6 months. The metabolic rate of diphenylhydantoin started with a half life of 6.1, 3.4 and 2.5 hours. A decrease in the metabolic rate of diphenylhydantoin to ½–⅓ after phenobarbital treatment was seen in two dogs, and an increase of 2–3 times in one dog.

Four dogs were given 3 mg/kg/day of phenobarbital and 10 mg/kg/day of diphenylhydantoin. In this experiment, a transient increase in the metabolic rate of phenobarbital (of up to 100%), but no definite change in the metabolic rate of diphenylhydantoin was found. The plasma level of phenobarbital was found to be somewhat higher after 3 mg/kg/day of phenobarbital than after the same dose phenobarbital combined with 10 mg/kg/day of diphenylhydantoin. However, the individual variation was high, and the difference gradually subsided after treatment for 4–5 weeks.

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I. Introduction

During the last 15 years studies have shown that most drugs are metabolized in the liver, catalyzed by the microsomal enzymes Many physiological factors, and other drugs, affect the enzymes of the liver and hence also the biotransformation of drugs, both in experimental animals and in man

Enzyme activity is known to depend on temperature, remaining constant only within a closely defined thermal range In order to provide favourable conditions for their organic functions, homothermic animals strive to keep their body temperatures unchanged Only the large mammals, however, achieve this, whereas in the samll rodents, used mostly in laboratory experiments, body temperature changes with environmental temperature (e g, HERRINGTON 1940) Many drugs affect the mechanism regulating the body temperature Under the influence of narcotics in particular, small animals become almost poikilothermic (FUHRMAN 1947) Studies of drugs at different environmental temperatures have revealed that the drug response changes as body temperature is changed The effect of barbiturates has been found to be prolonged with changes in environmental or body temperature Most of the studies were based on measurement of the pharmacological effect There are surprisingly few studies reported in the literature dealing with the biotransformation of the drug as a function of environmental temperature or body temperature, as pointed out by MAYNERT (1965)

Against this background it seemed justifiable to study the effects of environmental temperature on tissue levels *in vivo* of two barbiturates which behave differently in the organism, viz pentobarbital and barbital, and furthermore, the effectiveness at different temperatures of the substances governing the amount and activity of enzymes catalyzing the decomposition of drugs

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II. Review of the literature

THE FATE OF PENTOBARBITAL IN THE ORGANISM

Pentobarbital, a short-term hypnotic, is a 5 ethyl 5 (L-methylbutyl) barbituric acid It is generally used both as a drug and in experimental studies The substance is rapidly and well absorbed on various methods of administration About 45 per cent of pentobarbital is bound to plasma albumin, the remainder being free (BRODIE et al 1953) After absorption it is rapidly distributed into the tissues, fairly uniformly, although the highest content is noted in the liver According to BRODIE (et al, 1953), the levels in the kidney, brain and plasma were lower

The metabolism of the different barbiturates occurs primarily in the liver This has been proved by producing a liver lesion with hepatotoxic substances, such as ethionine, carbon tetrachloride, yellow phosphorus, and radioactive phosphate (NEUBERT 1957, HERKEY et al 1958, MAIBAUER et al 1958, NEUBERT & MAIBAUER 1959), which prolonged the pharmacological action of barbituric acid derivatives other than barbital The metabolism of pentobarbital occurs mainly through side-chain oxidation (BRODIE et al 1953) Using pentobarbital labelled with radioactive carbon, a total of 9 metabolites have been detected, most of them composed of either pentobarbital carboxylic acid (TITUS & WEISS 1955) or pentobarbital alcohol The latter exists in the form of two diastereoisomers (MAYNERT & DAWSON 1952) Pentobarbital carboxylic acids and pentobarbital alcohols occur in equal quantities (COOPER & BRODIE 1957) Ring cleavage occurs in 2—3 per cent (TITUS & WEISS 1955), and about 1 per cent is excreted unchanged A small number of the metabolites are as yet unidentified

Hydroxylation of pentobarbital and the additional oxidation of the primary alcohols formed occurs, catalyzed by several enzymes, in the microsomal fraction of the liver (COOPER & BRODIE 1957) The reactions require, in addition to molecular oxygen, reduced triphosphopyridine nucleotide No specific microsomes or soluble enzymes taking

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FACTORS AFFECTING DRUG METABOLISM

The amount and activity of enzymes catalyzing the biotransformation of drugs is subject to continuous change even in the same individual The change may be spontaneous, and also numerous physiological and non physiological factors may influence it The result is changed biotransformation of the drugs (FOUTS 1962)

The following factors have been found to affect the metabolism of drugs in the liver

Animal species and strains Biotransformation of drugs varies from one species to another

QUINN et al (1958) found that the half lives of hexobarbital, antipyrine and anilide in the mouse, rat, guinea pig, rabbit, dog and man differed considerably The activity of azoreductase varies noticeably between the species (FOUTS et al 1957 b) In addition to variations in the biotransformation of drugs between species, differences have also been noted between the strains of the same species (JAY 1955, QUINN et al 1958) In man, distinct differences exist between individuals in the half life of drugs (e g BRODIE et al 1949, BURNS et al 1953, PETERS 1960)

Age Drug metabolism varies with age, detailed studies have been carried out on experimental animals In a newborn animal, the liver is incapable of metabolizing foreign matter (JONDORF et al 1958) This is because the necessary stimulus for the development of liver enzymes is absent in the foetal period, and therefore enzyme activity cannot develop (FOUTS 1962) Neither the biotransformation of foreign matter nor the metabolic functions occurring normally, such as conjugation of bilirubin into glucuronide, are fully developed (BROWN A K et al 1958, SCHMIDT et al 1959, INSCOE & AXELROD 1960) The ability of laboratory animals to metabolize drugs to any significant degree commences at the age of two weeks, while metabolic activity does not reach the adult level until the animal is 4—8 weeks old (JONDORF et al 1958, FOUTS & ADAMSON 1959)

Food The quality and quantity of food and liquid consumed by the animals have been found to affect the biotransformation of drugs BROWN, R R et al (1954) found that the quality of food influences the capacity of animals to metabolize drugs In guinea pigs and pigs suffering from scurvy the toxicity of procaine and pentobarbital increases (RICHARDS 1941, 1947, CONNEY et al 1961) and hydroxylation of acetanilide is slowed down (AXELROD et al 1954 b) Fasting has a retarding effect on the biotransformation of

part in metabolism have been isolated It seems probable that the enzymes are non specific and catalyze several reactions simultaneously (BRODIE et al 1958) Through oxidation and hydroxylation the barbiturates become more polarized and more water soluble which makes it easier for the kidneys to remove them from the blood (REMMER & MERKER 1963)

None of the metabolites of pentobarbital has been found to possess pharmacological action (BRODIE et al 1953) Since pentobarbital furthermore, is almost completely metabolized before it leaves the organism, it is well adapted for studies attempting to reveal the influence of temperature on drug metabolism of the liver

THE FATE OF BARBITAL IN THE ORGANISM

Barbital is a 5 5 diethyl barbituric acid It is a drug of prolonged action, used as hypnotic since 1903 (FISCHER & VON MEERING 1904) The action of barbital starts slowly (in 14—15 minutes) even on intravenous administration The mode of administration does not affect its action Barbital is well absorbed negligibly bound to the plasma albumin and distributed uniformly into the tissues, it is for example, found on intravenous administration in the gastric and pancreatic juices (KOFFEY et al 1940), and in foetal tissue (DILLE 1934) The highest concentrations of barbital are noted in renal and brain tissue (DILLE et al 1935) The distribution of barbital throughout the brain is uniform (VOGT 1935)

The 5 , 5 diethyl side-chain is resistant to oxidative decomposition For this reason barbital was considered completely non metabolized (e g , MAYNERT & VAN DYKE 1949) More recent studies have however, revealed that two to five per cent of barbital is metabolized in dog (MAYNERT & VAN DYKE 1950) rat and guinea pig (BURNS et al 1957) Recorded metabolites are 5 ethylbutyl barbituric acid 5 ethyl 5 beta hydroxy ethyl barbituric acid and the glucuronide of the latter (EBERT et al 1964)

Barbital is excreted into the urine during several days In man 70 per cent is excreted in 5 days but the excretion continues up to 2—3 weeks (FISCHER & VON MEERING 1904) In animals barbital is excreted somewhat more quickly about 50 per cent in 24—48 hours but the excretion continues up to 7 days (REINERT 1928 SCHLOSSMAN 1933)

Owing to its slight, slow decomposition barbital for the purposes of short term experiments can be considered a drug almost totally exreted into the urine

liver tumour tissue, either primary or transplanted (FOUTS et al 1961) After subtotal hepatectomy, oxidative and reductive activities are absent from the regenerating tissue (FOUTS et al 1961) These activities are not normalized in the regenerating liver until that regeneration is completed, even if dealkylation of codeine has been seen during the regenerative phase (FOUTS et al 1961) On the other hand, an operative measure alone, for example laparotomy, retards the oxidation of hexobarbital and chlorpromazine (VON DER DECKEN & HULTIN 1960) Human cirrhosis of the Laennec type was not found to change appreciably the decomposition of drugs (BRODIE et al 1959)

Other drugs Many substances, if given in adequate total doses, have been found to stimulate not only their own metabolism in the organism but also the biotransformation of other substances of a completely different structure It has been seen that substances stimulating drug metabolism induce the synthesis of the microsomal enzyme proteins of the liver (CONNEY & BURNS 1962, REMMER 1962, CONNEY 1965) RICHARDSON et al (1952) were the first to give attention to the matter, they found that after a potent liver carcinogen, 3 methyl-4 diethyl aminoazobenzene, the formation of hepatoma was inhibited in a rat which was simultaneously given 3 methylcholantrene This latter induces microsomal enzymes of the liver, upon which amino azo dyes are rapidly decomposed into harmless metabolites and hepatoma formation is inhibited (CONNEY et al 1956) Long acting barbiturates have also been found to induce microsomal enzymes REMMER 1959 a b, CONNEY & BURNS 1959, CONNEY et al 1960) A characteristic of enzyme induction caused by the drugs is its slow development, in rats pretreated with phenobarbital, for example, an accelerated biotransformation of hexobarbital is noted after 12 hours, yet the effect does not reach its maximum until 48 hours, returning to its normal level in 5—7 days (REMMER 1959 a, b) Investigations in recent years have revealed more than 100 substances which stimulate the biotransformation of some other agent by increasing the amount of microsomal enzymes which metabolize drugs in the liver (CONNEY 1965)

No correlation has been traced between the pharmacological effect and the structure of these substances stimulating drug metabolism, but the majority of them are fat soluble within the physiological pH range The stimulation of drug metabolism depends on the dose and the duration of the effect, long acting substances are usually better inducers than short acting (REMMER & SIEGERT 1962)

many substances (DIXON et al 1960) The glycogen content of the liver has been found to fall during fasting, and this change correlates with the changed biotransformation of drug (FOUTS 1965) In animals that have fasted, the enzyme activities are not restored to their original level until 48 hours after feeding restarted (DIXON et al 1960) *Restricted liquid for 24 hours shortens, whereas lasting dehydration* prolongs the duration of sleep induced by hexobarbital (BORGELLECA & MANTHEI 1957)

Hormone balance Certain hormones have been found to affect drug metabolism The part played by sex hormones in the drug metabolism of the rat was discovered by HOLCK et al (1937) who found that *male rats were more resistant than female rats to the toxic effect* of several drugs This has been demonstrated by giving female rats testosterone (QUINN et al 1958, REMMER 1958 a, INSCOE & AXELROD 1960) which accelerated their drug metabolism Oestrogen given to male rats, on the other hand, retards the decomposition of drugs (QUINN et al 1958, REMMER 1958 a) *It is remarkable that in other animals no similar correlation of biotransformation of drugs to sex hormones* has been demonstrated (HOLCK et al 1937, REMMER 1958 a, QUINN et al 1958) *The hormones of the adrenal cortex affect the metabolism of drugs* The period of pharmacological action of hexobarbital (REMMER 1958 a, b) and morphine (WAY & ADLER 1960) *is extended in adrenalectomized rats* Substitution therapy with either cortisone or prednisolone, but not with mineralocorticoids, *raises drug metabolism in adrenalectomized animals* above the control level (REMMER 1957, 1958 b) Thyroxin stimulates general metabolism and reduces the glycogen content of the liver (COCHIN & SOKOLOFF 1960), in this case the inhibited decomposition of drugs is comparable to the state that follows fasting Prolonged activity of methadone (SUNG & WAY 1953) and hexobarbital (COCHIN & SOKOLOFF 1960) has been found in animals pretreated with thyroxin *By contrast the activity of zoxazolamine was found to be shortened in rats treated with thyroxin* (CONNEY & GARREN 1961)

Liver diseases In some liver diseases the enzymes catalyzing drug metabolism are decreased and, as a result the biotransformation of drugs is retarded *Biliary duct obstruction of short duration has no adverse effect on enzyme activity* (MCLUEN & FOUTS 1961), whereas obstruction of a long duration results in the formation of desoxycholic acid, which destroys endoplasmic vesicles of the liver cells causing a fall in the activity of oxidative enzymes (PALADE & SIEKIEWITSCH 1956) Neither oxidative nor reductive enzyme activities are seen in

incubation temperature (rat liver preparation) was +37°C If the temperature was raised or lowered beyond this thermal range, the N methylation of morphine, N and O methylation of codeine, and hydroxylation of ethyltryptamine were retarded RINK et al (1956) studied the effect of temperature on the half life of morphine and thiopenthal in a liver perfusion test They found that a temperature fall, from +37°C to +24°C, extended the half life of morphine from 94 minutes to 317 minutes and that of thiopenthal from 46 minutes to 537 minutes

In vivo investigations on the other hand, have given surprisingly little attention to the part played by environmental temperature in drug metabolism (MAYNERT 1965) This is the more surprising as temperature change has been found to produce many kinds of changes in the activity of the organism (JOHNSON et al 1963)

The small rodents most commonly used in laboratory experiments are homothermic, yet the environmental temperature does affect their body temperature (HERRINGTON 1940, DEPOCAS 1957, USINGER 1957, FUHRMAN & FUHRMAN 1961) Studies have shown that the body temperature remains unchanged only within a given range of environmental temperature, »thermal neutrality» which for guinea pig, rat and mouse is +30 +31°C, +28 +29°C, and +31-+33°C, respectively In these temperature ranges the calorie consumption per 24 hours is also lowest in these animals (HERRINGTON 1940) A rise or fall in temperature beyond the thermal neutrality range is accompanied by a distinctly increased calorie consumption, the animal striving to keep its body temperature unchanged to provide constant conditions for its organic functions HERRINGTON (1940) studied in detail the calorie consumption and body temperature of guinea pigs, mice and rats at different environmental temperatures In a cold environment the metabolism of all test animals increased smoothly, a fall in body temperature does not start until the limits of the physiological regulation range are reached In a hot environment, however, considerable individual differences are noted in the increase of calorie consumption and in body temperature This was attributed to poor thermal regulation in a hot environment Some animals moistened their skins with their secretions and so improved the evaporation of heat so that body temperature should not rise Not all animals could do this, and the result was a rise in their body temperature

When the effect of drugs is studied in animals whose body temperature depends on environmental temperature, the response produced by the drug must be correlated to body temperature, since otherwise

In pretreated animals proliferation has been noted in the smooth membrane of the liver cell a change that correlates with the accelerated biotransformation of the drug (REMMER & MERKER 1963) Although acceleration in enzyme action has been clearly demonstrated it has been impossible to isolate these enzymes (CONNEY 1963) REMMER 1962)

Apart from the inducing effect some drugs have been found to exert an inhibiting effect on the biotransformation of an agent of a completely different structure This is based on the inhibition of the activity of the microsomal enzymes of the liver The best known and most used agent inhibiting enzymatic drug metabolism is SKF 525 A beta dimethylaminoethyl diphenyl propylacetate (NETTER 1962) The first observations of the inhibiting effect of this agent on drug metabolism date back to 1954 55 when COOK et al (1954 a b) showed that it prolonged the duration of sleep induced by hexobarbital *in vivo* The inhibiting action was noticed immediately on administration of SKF 525 A while the most effective inhibition was produced when the agent was given 40 60 minutes before the tested drug It was later noted that this also occurs *in vitro* (AXELROD et al 1954 a) Pretreatment with SKF 525 A prolongs the oxidation of barbiturates (COOK et al 1954 a b) deamination (COOPER et al 1954) some reductions (FOUTS & BRODIE 1957 b) and hydrolytic reactions (NETTER 1959)

In recent years numerous other agents have been found to inhibit biotransformation of drugs in the same way as SKF 525 A they prolong the pharmacological action of other agents both *in vivo* and *in vitro* (reviews e g by NETTER 1962 KATO et al 1964) Among them are the monoamino oxidase inhibitors (FOUTS & BRODIE 1957 a HOLZ et al 1957 LAROCHE & BRODIE 1960 KATO et al 1964) The inhibition of monoamino-oxidase and the inhibition of the activity of enzymes participating in the biotransformation of drugs are two separate properties The former is achieved even with small doses whereas the latter requires a large quantity of the inhibitor (LAROCHE & BRODIE 1960 NETTER 1962) The most effective inhibitor of biotransformation of drugs among monoamino oxidase inhibitors is JB 516 (pheniprazine) (NETTER 1962 KATO et al 1964)

TEMPERATURE AND DRUG METABOLISM

Drug metabolism catalyzed by the microsomal enzymes of the liver has been much studied in recent years *in vitro* Close control of temperature has been found imperative to retain enzyme activity LEADBEATER & DAVIES (1964) found that the optimal

incubation temperature (rat liver preparation) was +37°C If the temperature was raised or lowered beyond this thermal range, the N methylation of morphine, N and O methylation of codeine, and hydroxylation of ethyltryptamine were retarded RINK et al (1956) studied the effect of temperature on the half life of morphine and thiopenthal in a liver perfusion test They found that a temperature fall, from +37°C to +24°C, extended the half life of morphine from 94 minutes to 317 minutes and that of thiopenthal from 46 minutes to 537 minutes

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When the effect of drugs is studied in animals whose body temperature depends on environmental temperature, the response produced by the drug must be correlated to body temperature, since otherwise

the results obtained will be contradictory (FUHRMAN & FUHRMAN 1961) Besides the correlation of body temperature to environmental temperature, it should be borne in mind that many drugs affect the thermal regulation of the body, with the result that the effect of environmental temperature on the body temperature is emphasized The influence of drugs on the thermal regulation of the body depends on the structure and the mechanism of the action of the drug (VON EULER 1961) Under the influence of narcotizing drugs, such as barbiturates, the small laboratory animals become almost poikilothermic since the drug paralyzes their thermal regulation (FUHRMAN 1947) BRUNTON, as early as 1874, found it important to keep the body temperature within the normal range while he studied the drug effect in different environmental temperatures He found that guinea pigs treated with toxic doses of chloral hydrate recovered if their body temperatures were not allowed to fall In later studies of drug effect at different temperatures, attention has usually been given only to environmental temperature, and the altered drug response correlated to it It was found that the duration of sleep produced by short acting barbiturates was extended in cold environment, whereas no similar prolongation of sleep was noted with long acting barbiturates, such as barbital and phenobarbital (e g, RAVENTOS 1938, CAMERON 1938, GAYLORD & HODGE 1944, KOMLOS & FOLDES 1959) BARCELLECA & MANTHEI (1957) analysed in greater detail the effect of environmental temperature on the duration of sleep produced by hexobarbital They found that the decisive environmental temperature limit where extension of the duration of sleep began, fell between +22-+26°C In a room with a temperature below +22°C the duration of sleep in rats treated with hexobarbital was significantly longer than in those kept at temperatures ranging from +28 to +32°C

The role of body temperature in studies of the duration of barbiturate effect in different environmental conditions was actually brought to the fore by FUHRMAN in 1947 He used in his experiments mice whose body temperatures were reduced by cooling to +27 C and control animals whose temperatures were kept at +37°C The duration of sleep induced with pentobarbital and allyl isobutyl barbituric acid was extended in mice with the lower body temperature, whereas these animals exhibited no similar extension in the duration of sleep induced with barbital The barbiturate levels in tissues, however, were not analysed FUHRMAN considerad that the extended duration of sleep in mice whose body temperatures were reduced to +27°C was attributable to retarded catabolism of the drug which he assumed to

occur enzymatically The explanation, however, is ambiguous, since environmental temperature has been found to affect the distribution of barbiturates in different tissues (SETNIKAR & TEMELCOU 1962) In addition, a small dose of pentobarbital has been found to induce sleep of longer duration in hot than cold environment After a large dose of pentobarbital this is reversed (SHAW & SHANKLEY 1948) This would suggest that the barbiturate action is somewhat different at different temperatures Hence the measuring of the duration of sleep does not provide the correct basis for the study of the biotransformation of barbiturates, since other factors play a role For this reason analysis of tissue levels may be considered the method of choice for studying the influence of temperature on the biotransformation of barbiturates

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IV. Material and methods

ANIMALS

Male guinea pigs were used in the study The weights of the animals ranged from 350 to 450 grams Some 900 guinea pigs were used for the experiments All the animals came from the same breeder (The Yla Mankkaa Farm, Helsinki) and were bought in batches of 100 animals Before the experiments were started the animals lived for 4—7 days in stables, having free access to food and water

DRUGS AND CHEMICALS

The following agents were used for the study
— barbital sodium (Barbital Natrium, E Merck AG, Darmstadt)
— pentobarbital sodium (Medipolar OY, Oulu)
— phenobarbital sodium (Star OY, Tampere)
— pheniprazine hydrochloride (JB-516, Milwaukee Lakeside Wisconsin Laboratories, INC , USA)
— beta diethylaminoethyl diphenyl propylacetate (SKF 525 A, Smith Klein French, Philadelphia, USA)

Petrol ether and isoamyl alcohol were used for the extraction of the barbiturates Potassium dihydrogen phosphate, disodium hydrogen phosphate and sodium hydroxide pellets were used in the preparation of buffers All the chemicals were of analytical grade, supplied by E Merck AG Darmstadt

METHOD OF EXPERIMENT

The guinea pigs were weighed, placed in round bottomed metal wire cages (diameter 25 cm), one to a cage, and placed in rooms with automatic thermostats, in which the air flow and relative humidity were kept constant The following temperatures were used $+4 \pm 1$°C (cold), $+30 \pm 1$°C (neutral) and $+37 \pm 1$°C (hot)

III. The problems

The aim of the present work was to elucidate the influence of environmental temperature on the elimination of pentobarbital and barbital in guinea pigs in vivo by seeking to answer the following questions

1 How does environmental temperature affect the body temperature of the guinea pig strain used as test animals?

2 How does environmental temperature affect the fall of the tissue levels of pentobarbital and barbital, and is there any correlation with *the change in body temperature?*

3 What is the effect of enzyme inducers in different environmental temperatures on drug metabolism judged by the tissue levels of the barbiturates, and is there any correlation with recorded changes in body temperature?

4 What is the effect of enzyme inhibitors in different environmental temperatures on drug metabolism judged by the fall in the tissue levels of the barbiturates, and is there any correlation with recorded changes in body temperature?

against the pH 11 0 buffer with a spectrophotometer (Beckman DU spectrophotometer, wave length 240 mm) Every analysis included a water sample which, having passed through the method, gave a reading between 0 010—0 025 The liver and brain of the normal guinea pig gave readings between 0 040—0 065 and 0 040—0 060, respectively, these values were deducted from the readings for the barbiturate tissue levels Each series included in addition to the water sample, another sample to which a known amount of the barbiturate in question had been added, and the levels were calculated against this sample Of the pentobarbital added to the tissue, 92 per cent was recovered from the liver and 93 per cent from the brain, of the barbital added, 93 and 94 per cent, respectively Of the agents used for pretreatment, SKF 525 A and JB 516 injected into the animal did not raise the blank values if measured at 240 mμ, whereas pretreatment with phenobarbital raised the blank values of the liver and brain as follows the tissue reading after 48 hours for guinea pigs pretreated with a small dose of phenobarbital (10 mg/kg) did not differ from the reading for the tissue of control animals In the guinea pigs treated with a larger dose of phenobarbital (50 mg/kg) the liver and brain tissues were found to give at the cold temperature readings which corresponded to 6 7 μg/g and 5 2 μg/g After 72 hours at this temperature the values were 2 5 and 1 9 μg/g The readings obtained after 48 hours for liver and brain tissues of guinea pigs kept at the hot temperature corresponded to 2 5 and 2 0 μg/g At the neutral temperature a 50 mg/kg premedication dose of phenobarbital was found to raise the liver and brain tissue readings in the same way as in the control animals After a larger dose (70 mg/kg) the liver and brain tissues of guinea pigs kept at the cold temperature were found to give readings identical to those obtained after the smaller dose (50 mg/kg) At the neutral temperature the liver and brain tissues gave readings of 2 5 and 1.5 μg/g The above were mean values for the liver and brain tissues of 6 animals. In order to obtain only the readings given by pentobarbital and barbital in the tissues of animals pretreated with phenobarbital on spectrophotometer recording the above readings had to be subtracted

STATISTICAL TREATMENT

Statistical analysis, mean values, standard deviation, and standard error of the mean were calculated Student's t test was used to study the statistical difference between two mean values

Measurement of rectal temperature The guinea pigs' body temperatures were measured with an electrically operated thermometer, Universal Thermometer type TE 3 (Elektrolaboratoriet Ellab A/S, Copenhagen) Applicators (model RN 4), inserted 5 cm into the rectum beyond the anal sphincter, were used The meter was checked before and after temperature recording Temperatures were recorded at hourly intervals during 1—6 hours

Drug injections Drug injections were effected with a tuberculin syringe of 0 01 ml grading All the drugs were dissolved in distilled water and the following concentrations were used barbital sodium 100 mg/ml, pentobarbital sodium 20 mg/ml, phenobarbital sodium 10 and 35 mg/ml, SKF 525 A 10 and 50 mg/ml and JB 516 15 mg/ml The doses were calculated against the corresponding acid or alkali Phenobarbital sodium was injected under the skin of the back, the other injections were made from the guinea pig's right flank into the abdominal cavity Before injection, free movement of the needle in the abdominal cavity was obtained The doses given for premedication were phenobarbital sodium in cold and neutral environment 10, 50, and 70 mg/kg, in hot environment only 50 mg /kg, SKF-525 A 10, 50, and 100 mg/kg, and JB 516 15 mg/kg The dose of pentobarbital sodium, unless otherwise staded, was 30 mg/kg and that of barbital 250 mg/kg

Taking of tissue specimens At predetermined dates the guinea pigs were killed by a blow to the back of the neck, after which tissue specimens, the whole brain and a piece of 3—5 g of the liver, were taken quickly The specimens were placed in weighed glass decanters standing on ice, the decanters were closed tightly with aluminium foil after they had been weighed, and kept deep frozen (—20°C) until the chemical analysis was made

CHEMICAL ANALYSIS

Pentobarbital and barbital were analysed with the Brodie method (Brodie et al 1953 Smith 1961) Frozen tissue specimens were homogenized in a Potter Elvenhjelm type glass homogenizer in double volume of distilled water 2 0 ml of the homogenate was pipetted into 60 ml bottles fitted with ground glass stoppers To this was added 1 0 ml buffer of pH 5 5, 1 g sodium chloride and 40 ml petrol ether containing 1 5 per cent isoamyl alcohol The specimens were mixed in a shaker for 45 minutes and centrifuged 20 ml of the solvent phase was transferred into 60 ml glass stoppered bottles containing 4 0 ml buffer of pH 11 0 The barbiturate levels of the tissues were measured

against the pH 11 0 buffer with a spectrophotometer (Beckman-DU spectrophotometer, wave length 240 mm) Every analysis included a water sample which, having passed through the method, gave a reading between 0 010—0 025 The liver and brain of the normal guinea pig gave readings between 0 040—0 065 and 0 040—0 060, respectively, these values were deducted from the readings for the barbiturate tissue levels Each series included, in addition to the water sample, another sample to which a known amount of the barbiturate in question had been added, and the levels were calculated against this sample Of the pentobarbital added to the tissue, 92 per cent was recovered from the liver and 93 per cent from the brain, of the barbital added, 93 and 94 per cent, respectively Of the agents used for pretreatment, SKF-525 A and JB 516 injected into the animal did not raise the blank values if measured at 240 mμ, whereas pretreatment with phenobarbital raised the blank values of the liver and brain as follows the tissue reading after 48 hours for guinea pigs pretreated with a small dose of phenobarbital (10 mg/kg) did not differ from the reading for the tissue of control animals In the guinea pigs treated with a larger dose of phenobarbital (50 mg/kg), the liver and brain tissues were found to give at the cold temperature readings which corresponded to 6 7 μg/g and 5 2 μg/g After 72 hours at this temperature the values were 2 5 and 1 9 μg/g The readings obtained after 48 hours for liver and brain tissues of guinea pigs kept at the hot temperature corresponded to 2 5 and 2 0 μg/g At the neutral temperature a 50 mg/kg premedication dose of phenobarbital was found to raise the liver and brain tissue readings in the same way as in the control animals After a larger dose (70 mg/kg) the liver and brain tissues of guinea pigs kept at the cold temperature were found to give readings identical to those obtained after the smaller dose (50 mg/kg) At the neutral temperature the liver and brain tissues gave readings of 2 5 and 1 5 μg/g The above were mean values for the liver and brain tissues of 6 animals In order to obtain only the readings given by pentobarbital and barbital in the tissues of animals pretreated with phenobarbital on spectrophotometer recording, the above readings had to be subtracted

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curves of guinea pigs for 1—6 hours in cold, neutral and hot environments During the experiment the lowest and highest mean rectal temperatures were 38 2 38 4 38 8 39 1 and 39 9 40 5°C, respectively In the cold environment, the rectal temperatures were throughout within the normal range, in thermal neutrality there was an initial increase during the first hour, while later the mean value curve fell towards the initial level In the hot environment the rectal temperatures rose rapidly to the 40°C level and remained there throughout the period of the experiment

DISCUSSION

The guinea pigs used were able to maintain their body temperature in the cold environment for 6 hours The individual rectal temperatures differed little from the mean value HERRINGTON (1940) found that metabolism of the guinea pigs in cold environment increased evenly, and no dispersal occurred in body temperature between individuals Thermogenesis in the cold environment is equally divided between shivering and non shivering mechanisms (DAVIS 1963) As the animal adapts itself thermogenesis remains unchanged for up to 25 days, after which chemical thermogenesis assumes the whole responsibility for the retention of body temperature (DAVIS 1963) Guinea pigs kept in the warm environment on the other hand, were less able to maintain an even body temperature than those in the cold environment At the neutral temperature the rectal temperature rose initially, but in 6 hours the animals adapted themselves to the environment, and the rectal temperature fell to its initial level In the hot environment the body temperature rose rapidly to above 40°C and remained elevated throughout the 6 hours

The behaviour of the animals at different temperatures was revealing In the cold their coats bristled up, and they were restless In addition, they curled up into small balls In thermal neutrality they were calm and seemed to feel better In the hot environment they were at first excited and dashed about trying to get out of the cage When they were handled in the hot room the grip had to be firmer than at the lower temperatures The stay in the heat, however, had a soothing effect on the animals' behaviour, in 2—3 hours they tended to lie down on cage floors and appeared sluggish

V. Influence of environmental temperature on the guinea pig's rectal temperature

In earlier studies environmental temperatures have been found to affect the body temperature of the small laboratory animals The effects of the cold (+4°C), neutral (+30°C) and hot (+37°C) environment on the body temperature of guinea pigs used for the present study were studied for 1—6 hours in thermostatically controlled rooms by measuring the guinea pigs' rectal temperatures

RESULTS

At room temperature (+18—+22°C) the rectal temperatures of the guinea pigs ranged from 38 2 to 38 4°C Fig 1 gives the mean value

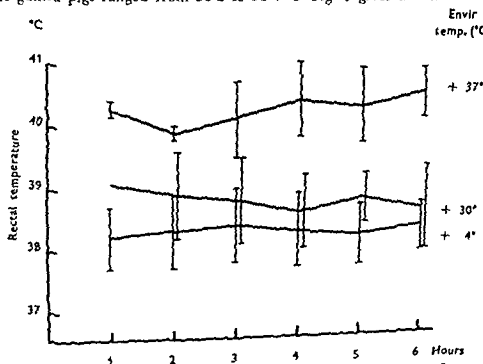


Fig 1 Effect of environmental temperature on guinea pig's rectal temperature Six guinea pigs in each group Mean ± S E M

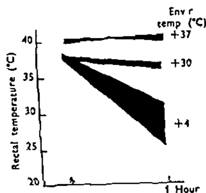


Fig 2

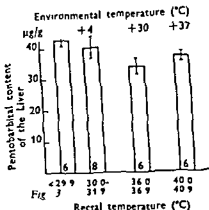


Fig 3

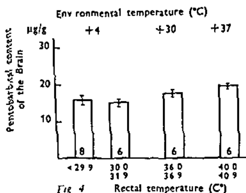


Fig 4

Figs 2 3 and 4 Effect of environmental temperature on guinea pig s rectal temperature and pentobarbital concentrat ons in the liver and brain The guinea pigs were for an hour in rooms at a given temperature, after which the pentobarbital was injected (30 mg/kg intraperitoneally) The blackened area indicates the range of rectal temperatures Pentobarbital concentrations in liver and brain are indicated by mean value columns ± S.E.M The numbers of animals are ind cated The guinea pigs are classified accor d ng to their rectal temperatures

Pentobarbital concentration in the brain The pentobarbital contents of the guinea pigs kept in hot and neutral environment were 19 3 ±3 6 μg/g and 17 5±2 1 μg/g In the guinea pigs in the cold environment with rectal temperatures above 30 0°C the concentration was 17 3±2 2 μg/g while for those whose rectal temperatures fell more markedly it was 18 2±2 8 μg/g The pentobarbital concentration in the brain was higher in hot environment than at the other temperatures though the difference was not statistically significant

2 hour experiment

Figs 5 6 and 7 shows the guinea pigs' rectal temperatures, and the pentobarbital concentrations in their liver and brain during the 2 hour experiment

Rectal temperature Two hours after the injection the rectal temperatures of the guinea pigs in the hot environment were 40 3-40 8°C, at the neutral temperature 35 6-37 6°C and in cold environment 16 5 28 2°C In the hot environment the rectal temperature remained station-

VI. Effect of environmental temperature on the fall in pentobarbital concentration in liver and brain tissue

The guinea pigs injected with pentobarbital (30 mg/kg) fell asleep in 3—5 minutes. Under barbiturate influence the effect of temperature on the animals' body temperature showed clearly. Considerable individual variation in the change of body temperature was recorded even between animals at the same temperature. For this reason the guinea pigs were divided into groups by the change in their rectal temperature.

RESULTS

1-hour experiment

Figs. 2, 3 and 4 give the guinea pigs' rectal temperatures, and the pentobarbital concentrations in liver and brain during the 1-hour experiment.

Rectal temperature. The rectal temperatures of the animals kept in the hot environment ranged from 40.1 to 40.7°C. At the neutral temperature the rectal temperatures fell to 36.0—36.9°C, and in the cold environment to 25.8—31.2°C. At the cold temperature, in particular individual dispersal in the change of rectal temperature was pronounced.

Pentobarbital concentration in the liver. The pentobarbital contents (the mean ± S.D.) in the liver of animals at the hot and neutral temperatures were 36.6 ± 3.6 µg/g and 33.1 ± 6.7 µg/g. In guinea pigs in the cold environment with rectal temperatures above 30.0°C the pentobarbital content of the liver was 39.8 ± 8.8 µg/g and in those that showed a more marked temperature fall the content was 41.6 ± 5.2 µg/g. A statistically significant difference in the pentobarbital concentrations in the liver was noted between the animals kept in thermal neutrality and those of the cold environment whose rectal temperatures fell below 29.9°C ($p < 0.01$).

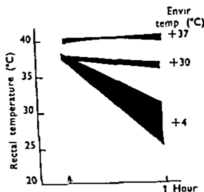


Fig 2

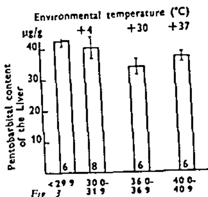


Fig 3

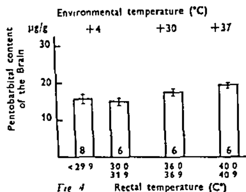


Fig 4

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2-hour experiment

Figs 5, 6 and 7 shows the guinea pigs' rectal temperatures, and the pentobarbital concentrations in their liver and brain during the 2 hour experiment

Rectal temperature Two hours after the injection the rectal temperatures of the guinea pigs in the hot environment were 40 3-40 8°C, at the neutral temperature 35 6-37 6°C and in cold environment 16 5-28 2°C In the hot environment the rectal temperature remained station-

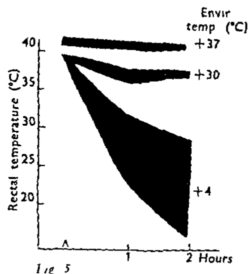


Fig 5

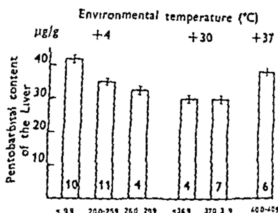


Fig 6 Rectal temperature (°C)

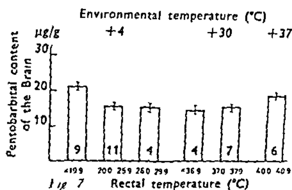


Fig 7 Rectal temperature (°C)

Figs 5 6 and 7 Effect of environmental temperature on the guinea pigs rectal temperature and the pentobarbital concentrations in the liver and brain in a 2 hour experiment Other data as for Figs 2 3 and 4

ary at the elevated level, in thermal neutrality the fall in rectal temperature levelled off, and in the cold environment a marked fall in temperature continued in all the animals while individual dispersal increased

Pentobarbital concentration in the liver In guinea pigs in the hot environment the pentobarbital concentration in the liver was 37 9±4 8 μg/g At thermal neutrality the concentration was 27 7±2 1 μg/g for animals with rectal temperatures above 36 0°C, and 27 8±4 7 μg/g for those with rectal temperatures below 35 9°C For animals in the cold environment, with rectal temperatures above 36 0°C, the concentration was 32 4±5 3 μg/g, and for those with a heavier fall in temperature, to 20 0—25 9°C, and below 19 9°C, the concentrations were 35 0±4 1 μg/g and 42 0±4 8 μg/g respectively The effect of a sudden temperature fall on the fall in the pentobarbital concentration of the liver was studied in 5 animals by immersing them, immediately after the pentobarbital injection, in icewater The rectal temperature was reduced to 26 0±1 0°C and remained unchanged for 2 hours

The pentobarbital concentration in the liver was 47.0±2.0 µg/g which was higher than the liver concentration of the guinea pigs whose rectal temperatures fell slowly to 20.0—25.9°C.

A statistically significant difference was noted in the pentobarbital concentration in the liver between animals at thermal neutrality and those in the cold environment whose rectal temperature had fallen to or below 25.9°C ($p<0.05$ and $p<0.01$). Similarly, a statistically significant difference existed in liver concentrations between animals kept at thermal neutrality and in the hot environment ($p<0.05$).

Pentobarbital concentration in the brain. The pentobarbital concentrations in the brain of guinea pigs in the hot environment were 17.8±2.9 µg/g, at thermal neutrality 15.4±3.5 µg/g in animals with rectal temperatures exceeding 36,0°C, and 14.4±3.1 µg/g for those with lower rectal temperatures. In animals in the cold environment and with rectal temperatures exceeding 26.0°C the concentration was 15.4±3.9 µg/g, in those with rectal temperatures below 25.9°C it was 15.5±3.7 µg/g and in those with rectal temperatures below 19.9°C it was 20.9 µg/g. The pentobarbital concentration in the brain of the guinea pigs in thermal neutrality which had the lower rectal temperatures differed statistically significantly ($p<0.01$) from that of the guinea pigs in the cold environment whose rectal temperatures had fallen below 19.9°C.

3 hour experiment

Figs. 8, 9 and 10 show the guinea pigs' rectal temperatures and the pentobarbital concentrations in the liver and brain during the 3 hour experiment.

Rectal temperature. Three hours after the injection the guinea pigs' rectal temperatures were 40.1—41.2°C in the hot environment, 35.2—37.9°C at thermal neutrality, and 14.0—29.9°C in the cold environment. In the hot environment the rectal temperatures of some of the guinea pigs rose, at thermal neutrality they all rose towards the initial level, while in cold environment the heavy fall in rectal temperatures continued.

Pentobarbital concentration in the liver. In guinea pigs in the hot environment, with rectal temperatures above 41.0°C, the pentobarbital concentration in the liver was 25.8±2.6 µg/g, and in those with rectal temperatures below 40.9°C it was 25.8±2.6 µg/g. In the thermal neutrality range, the pentobarbital concentration in the liver of animals with rectal temperatures above 37.0°C was 20.5±6.5 µg/g, and in

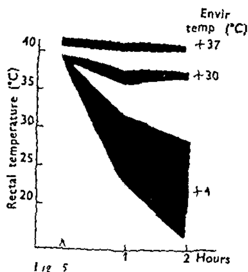


Fig 5

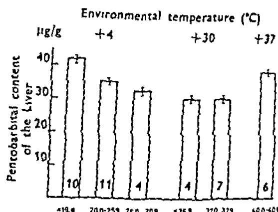


Fig 6

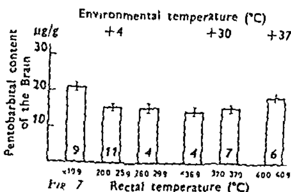


Fig 7

Figs 5 6 and 7 Effect of environmental temperature on the guinea pigs' rectal temperature and the pentobarbital concentrations in the liver and brain in a 2 hour experiment Other data as for Figs 2 3 and 4

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concentrations in the brain of those guinea pigs in the cold environment whose rectal temperatures had fallen below 26 0°C, differed significantly from the corresponding concentrations of the animals in hot and neutral conditions (the concentrations had remained higher, $p<0\ 05$ and $p<0\ 001$)

Change in guinea pigs' rectal temperature in the cold environment after different doses of pentobarbital Fig 11 shows the change in guinea pigs' rectal temperatures during 1—6 hours after varying pentobarbital doses in the cold environment The rectal temperatures of guinea pigs treated with large doses fell as heavily as those of the animals killed by a blow to the back of the neck After small doses the rectal temperature first fell distinctly but after 2—3 hours it rose to, and even above,the initial level

DISCUSSION

Rectal temperature Anaesthetics in narcotic doses suppress the thermal regulation mechanism of the body (HEMINGWAY 1941), and at the same time also paralyze the mechanism regulating the vascular tonus (THAUER 1942), making the small laboratory animal almost poikilothermic (FUHRMAN 1947) The dose of pentobarbital, 30 mg/kg, was sufficient to induce in guinea pigs a deep narcosis, so that their body temperature depended on the environmental temperature Environmental temperature had the smallest effect on guinea pigs kept

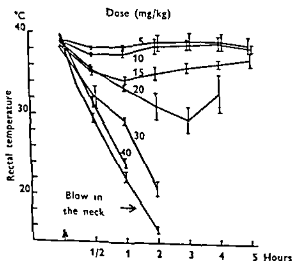


Fig 11 Effect of cold environment on the rectal temperature of guinea pigs treated with different doses of pentobarbital Six animals in each group Mean ± SEM

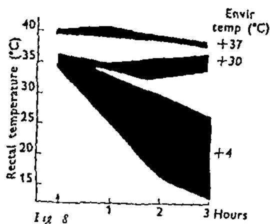


Fig 8

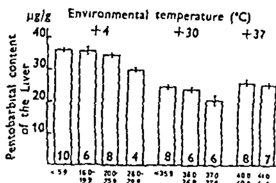


Fig 9 Rectal temperature (°C)

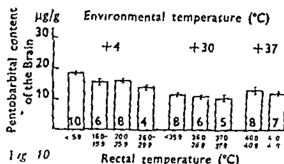


Fig 10

Figs 8 9 and 10 Effect of environmental temperature on the guinea pigs' rectal temperature, and the pentobarbital concentrations in liver and brain in a 3 hour experiment Other data as for Figs 2, 3 and 4

those with lower rectal temperatures the concentrations were 23 6 ± 2 5 µg/g and 24 5 ± 4 4 µg/g The pentobarbital concentration in the liver of the guinea pigs in the cold environment with rectal temperatures above 26 0°C was 30 5 ± 1 5 µg/g, while in those with lower rectal temperatures the concentrations were 34 8 ± 3 7 µg/g, 35 3 ± 4 4 µg/, and 36 0 ± 3 1 µg/g The pentobarbital concentrations in the liver of guinea pigs kept in the neutral and cold conditions differed significantly the concentration was highest at the neutral temperature and lowest in the cold environment ($p < 0\ 05$)

Pentobarbital concentration in the brain For guinea pigs in the hot environment and with rectal temperatures exceeding 41 0°C, the pentobarbital concentration in the brain was 13 3 ± 4 5 µg/g, while in those with lower rectal temperatures the concentration was 12 1 ± 2 5 µg/g At thermal neutrality, the animals with rectal temperatures above 37 0°C showed a brain concentration of 11 0 ± 1 7 µg g, while in those whose rectal temperatures had risen more slowly the corresponding concentrations were 11 5 ± 1 8 and 12 2 ± 1 8 µg/g The pentobarbital concentration in the brain of guinea pigs in the cold environment with rectal temperatures above 26 0°C was 13 6 + 1 7 µg/g, while in the animals with a lower rectal temperature the concentrations were 16 4 ± 2 4, 15 5 ± 3 1 and 17 2 ± 2 1 µg/g The pentobarbital

concentrations in the brain of those guinea pigs in the cold environment whose rectal temperatures had fallen below 26.0°C, differed significantly from the corresponding concentrations of the animals in hot and neutral conditions (the concentrations had remained higher, $p<0.05$ and $p<0.001$).

Change in guinea pigs' rectal temperature in the cold environment after different doses of pentobarbital. Fig. 11 shows the change in guinea pigs' rectal temperatures during 1—6 hours after varying pentobarbital doses in the cold environment. The rectal temperatures of guinea pigs treated with large doses fell as heavily as those of the animals killed by a blow to the back of the neck. After small doses the rectal temperature first fell distinctly but after 2—3 hours it rose to and even above, the initial level.

DISCUSSION

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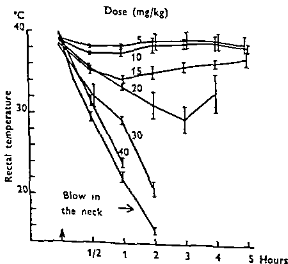


Fig. 11. Effect of cold environment on the rectal temperature of guinea pigs treated with different doses of pentobarbital. Six animals in each group. Mean ± S.E.M.

at thermal neutrality The rectal temperature fell distinctly during the first hour During the second hour the fall in rectal temperature levelled off, and during the third hour it rose towards the initial level In the thermally neutral environment the thermal regulation of the guinea pig is at its minimum (HERRINGTON 1940) and even under the influence of pentobarbital the remaining thermogenesis suffices to offset the loss of heat so that the rectal temperature does not fall much It has been demonstrated that thermogenesis exists even in deep narcosis, though heavily reduced (WINNIE 1964) Individual dispersal in rectal temperature occurred during the third hour when body temperatures of all the guinea pigs rose, most markedly in those with the lowest pentobarbital concentrations in liver and brain It may be concluded therefore, that in the guinea pigs whose rectal temperatures were best capable of resuming their initial level, the pentobarbital decomposing capacity of the liver was the most developed Earlier studies have revealed considerable differences in the duration of sleep in guinea pigs after an intraperitoneal injection of pentobarbital (CARMICHAEL & POSEY 1937), however, the guinea pigs' body temperatures were not recorded, nor was the pentobarbital concentration in the tissues analysed It would seem natural, however, that in guinea pigs with longer duration of sleep the decomposition of pento barbital in the liver was slower, since the duration of drug action is correlated with the dose Furthermore, earlier investigations have shown that animals with best developed general metabolism feel best in the cold environment (GOESSLIN 1949, MOUNT 1963), agreeing with the results of the present study

The decomposition of pentobarbital in guinea pigs shows individual variation correlated to the change in body temperature under the influence of the drug In the cold environment the thermogenesis of guinea pigs subject to pentobarbital action was insufficient to replace the increased thermal loss the rectal temperatures of all animals fell heavily The individual dispersal in the fall of rectal temperature was even more pronounced than under thermally neutral conditions According to the results the fall in the tissue concentrations of pentobarbital correlated with the change in body temperature down to 26°C a limit where the enzymatic decomposition of pentobarbital *in vivo* apparently ceased and a heavier fall in body temperature no longer played any part as inhibitor of the biotransformation of pentobarbital The rectal temperature of guinea pigs in the hot environment exceeded 40°C three hours from injection In smaller animals such as mice, a fall in body temperature has been shown to occur even at this temperature (WINNIE 1964) During sleep the guinea pigs did not

actively moisten their skins, as HERRINGTON (1940) had observed, and the rectal temperature in the hot environment was relatively uniform

The fall in rectal temperature was initially correlated to the dose of pentobarbital administered (Fig 11) After 2—3 hours the rectal temperature of animals treated with small doses of pentobarbital rose to, and even above, the initial level A similar rise in body temperature under the action of pentobarbital has been found in larger animals too, such as cat and dog (EKSTROM 1951, VON EULER & SODERBERG 1958) This has been tentatively attributed to a rise in the thermal regulation level (VON EULER 1961) In animals treated with large doses, on the other hand, there was a sharp uniform drop in rectal temperature nearly as sharp as in the animals killed by a blow to the neck

Pentobarbital concentration in the liver According to the results (Figs 3, 6 and 9) the environmental temperature affects the rate of fall in the pentobarbital concentration of the liver Of decisive importance is the change in body temperature during drug action This is because the change in rectal temperature under the influence of pentobarbital is not the same in all guinea pigs even though the environmental temperature is the same, dispersal occurs in the rise or fall of rectal temperature Both the rise and the fall of body temperature had a retarding effect on the disappearance of pentobarbital from liver tissue

The decomposition of pentobarbital was fastest in guinea pigs kept in thermally neutral conditions But even at this temperature the biotransformation of pentobarbital was not of the same intensity in all animals, and individual differences were seen, correlated to body temperatures The present results agree with the findings reported in the literature, according to which decomposition of drugs *in vitro* is fastest at the +37°C level, while a fall in temperature has a retarding effect (RINK et al 1956 LEADBEATER & DAVIES 1964) Furthermore, earlier studies have shown that the acute toxicity of pentobarbital to the rat is lowest close to the neutral temperature range, but increases steeply if environmental temperature is lower (KEPLINGER et al 1959)

The retarding influence of the cold environment on the biotransformation of pentobarbital emerged within an hour It became more marked during the second hour, when the body temperature of most of the animals fell to 26°C Although the change in body temperature in the cold environment in animals under the influence of pentobarbital was pronounced, decomposition of pentobarbital nevertheless continued in the liver This is shown by the fact that in guinea pigs, whose rectal temperatures were suddenly lowered to 26°C, the pentobarbital

concentration in the liver had remained at a higher level than in the animals whose rectal temperatures, due to the drug, slowly fell to the same level The decomposition of pentobarbital ceases at 26°C *in vivo*, this in indicated by the fact that in guinea pigs whose rectal temperatures in the 3-hour test fell to the 16°C level the pentobarbital content in the liver was the same as in animals with a rectal temperature 10°C higher

In guinea pigs in the hot environment the biotransformation of pentobarbital in liver tissue was distinctly retarded as compared with animals kept in thermally neutral conditions The body temperature rose in this environment 2—3°C above the normal level The biotransformation of drugs in the hot environment has not been studied earlier *in vivo* by measuring the drug concentrations in the tissues The present results, however, agree with observations made *in vitro*, according to which the decomposition of drugs is retarded as temperature rises above +37°C (LEADBEATER & DAVIES 1964) and acute toxicity undergoes changes when environmental temperature rises (KEPLINGER et al 1959)

SETNIKAR & TEMELCOU (1962) found that the fall in the blood level of pentobarbital, but not of barbital, in the dog was correlated to environmental temperature The study was concerned only with blood levels of animals kept in thermal neutrality or in the cold environment The only study reporting on drug decomposition correlated to body temperature is by HEINONEN (1966), on the metabolism of lidocaine The tissue levels of lidocaine metabolized in the liver were not found to correlate to body temperature or to any change in body temperature under the influence of the drug a finding contradictory to the present results

Pentobarbital concentration in the brain Pentobarbital is metabolized only in liver tissue (BRODIE et al 1953), and hence the pentobarbital concentration in the brain reflects, passively the biotransformation in the liver On the other hand, pentobarbital has been found to enter the brain tissue of the rat faster in hot than cold environment (SETNIKAR & TEMELCOU 1962), which implies that temperature changes the distribution of pentobarbital in the tissues Figs 4, 7 and 9 reveal that the pentobarbital concentration of the brain shows a disposition towards a higher level with increasing temperature The present results explain the observation by SHAW & SHANKELY (1948), according to which 'rats treated with a small dose of barbiturates sleep longer in a hot than cold environment, whereas those treated with a large dose of barbiturate sleep longer in cold than hot environ

ment." In animals treated with a small dose of barbiturate the decomposition of the drug in the liver takes place at the same rate under hot and cold conditions, since the cold environment cannot, in the short period of time, induce any change in the body temperature of the animals affected by the drug large enough to slow down the biotransformation of the drug in the liver The rise of body temperature in the hot environment is a quicker reaction in the small animals than the fall of body temperature in a cold environment In animals whose body temperature rises the biotransformation of pentobarbital in the liver tissue is slowed down, and the duration of drug action, i e the duration of sleep after an intraperitoneal injection, is prolonged But in animals treated with a large dose of barbiturate in a cold environment the body temperature falls heavily, which again slows down the biotransformation of the drug more than does an increase of body temperature due to an environmental temperature exceeding the thermally neutral range

It is apparent that while body temperature rises, the action of barbiturates in the brain tissue undergoes some change, since animals have been found to wake up with a higher barbiturate concentration in the brain in a hot than in a cold environment (WINNIE 1964) Not all animals sleeping at the same temperature, however, wake up when the drug concentration falls below a certain level, on the contrary, there is a considerable degree of individual variation (SMITH 1961) For this reason, in studies of drug metabolism *in vivo in different* environmental temperature conditions, the duration of sleep *cannot* be used to indicate the rate of biotransformation, the barbiturate concentrations in the tissues must be *analysed*

concentration in the liver had remained at a higher level than in the animals whose rectal temperatures, due to the drug, slowly fell to the same level. The decomposition of pentobarbital ceases at 26°C *in vivo*, this in indicated by the fact that in guinea pigs whose rectal temperatures in the 3-hour test fell to the 16°C level the pentobarbital content in the liver was the same as in animals with a rectal temperature 10°C higher.

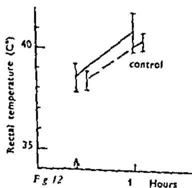
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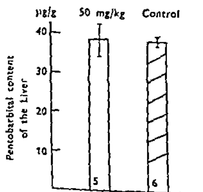
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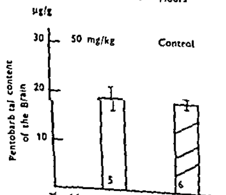
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Pentobarbital concentration in the liver In the hot environment the pentobarbital concentration in the liver of pretreated animals was 38 9±6 0 μg/g and in that of the control animals 38 8±2 0 μg/g In thermal neutrality and in the cold environment the lowest pentobarbital concentrations in the liver were seen in guinea pigs pretreated with 50 mg/kg, viz 30 3±3 7 μg/g and 20 2±3 9 μg/g Animals pretreated with a larger or a smaller dose showed higher liver concentrations The relevant concentrations for control animals were 35 5±2 4 μg/g and 35 5±3 5 μg/g

Pentobarbital concentration in the brain In hot environment the pentobarbital concentration in the brain of the pretreated animals was 19 8±3 5 μg/g and in that of the control animals 18 8±2 0 μg/g In thermal neutrality and in the cold environment the concentrations in guinea pigs pretreated with 50 mg/kg were 17 4±6 1 and 17 0±3 6 μg/g At neutral temperature the concentrations of guinea pigs pretreated with the largest and the smallest dose were the same, whereas in the cold environment they were higher in these groups







Figs 12 13 and 14 Rectal temperatures of guinea pigs pretreated with phenobarbital (50 mg kg subcutaneously) and of the corresponding control animals in the hot environment and the pentobarbital concentrations in their liver and brain one hour after pentobarbital injection The pentobarbital (30 mg/kg) was injected 48 hours after pretreatment. Mean ± S E M The number of animals is indicated

VII. Effect of enzyme-inducer (phenobarbital) at different temperatures

Phenobarbital was chosen for the present study as an agent to induce the microsomal enzymes of the liver, because it is the best known and most widely used agent in comparable studies. The guinea pigs were kept for an hour in the hot rooms, after which the inducing agent was injected. This pretreatment was followed, after 48, 72 or 120 hours, by an intraperitoneal injection of pentobarbital. The criteria indicating the intensity of the induction effect were the change in the rectal temperature of the pretreated animals under the influence of pentobarbital, and the analysis of pentobarbital concentrations in the liver and brain from specimens taken one hour after pentobarbital injection.

RESULTS

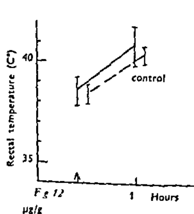
The results are presented in Figs. 12—20, which give the rectal temperatures of the pretreated guinea pigs and the corresponding control animals, and the pentobarbital concentrations in the liver and brain samples of the same animals, taken one hour after pentobarbital injection.

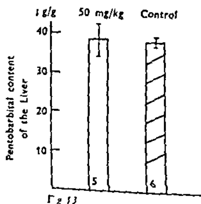
Rectal temperature. In hot environment the rectal temperature of the pretreated animals rose under the influence of pentobarbital to 40.2—42.0°C and that of the control animals to 39.8—41.2°C. At thermal neutrality the rectal temperature of the pretreated animals fell to 36.1—37.7°C and that of the control animals to 34.1—37.4°C. In the cold environment the temperatures were 28.6—36.1°C and 28.9—31.6°C respectively. At thermal neutrality and in the cold environment the fall in rectal temperature due to pentobarbital effect was smallest for those pretreated with 50 mg/kg. In animals pretreated with a smaller (10 mg/kg) and with a larger (70 mg/kg) dose the fall in rectal temperature was heavier.

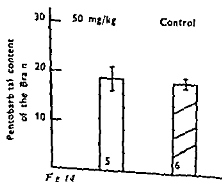
A preliminary communication on the subject was made at the First Medical Meeting (Lääketieteen päivät) Helsinki March 31—April 1 1967. »Enzyme induction due to phenobarbital in different environmental temperatures» (E. SOTANIEMI & N. KÄRKI)

Pentobarbital concentration in the liver In the hot environment the pentobarbital concentration in the liver of pretreated animals was 38 9±6 0 μg/g and in that of the control animals 38 8±2 0 μg/g In thermal neutrality and in the cold environment the lowest pentobarbital concentrations in the liver were seen in guinea pigs pretreated with 50 mg/kg viz 30 3±3 7 μg/g and 20 2±3 9 μg/g Animals pretreated with a larger or a smaller dose showed higher liver concentrations The relevant concentrations for control animals were 35 5±2 4 μg/g and 35 5±3 5 μg/g

Pentobarbital concentration in the brain In hot environment the pentobarbital concentration in the brain of the pretreated animals was 19 8±3 5 μg/g and in that of the control animals 18 8±2 0 μg/g In thermal neutrality and in the cold environment the concentrations in guinea pigs pretreated with 50 mg/kg were 17 4±6 1 and 17 0±3 6 μg/g At neutral temperature the concentrations of guinea pigs pretreated with the largest and the smallest dose were the same, whereas in the cold environment they were higher in these groups







Figs 12 13 and 14 Rectal temperatures of gu nea p gs pretreated with phenobarb tal (50 mg kg subcutaneously) and of the correspond ng control animals in the hot environment and the pentobarb tal concentrations in the r l ver and bra n one hour after pentobarb tal inject on The pentobarb tal (30 mg/kg) was injected 48 hours after pretreatment Mean ± S E M The number of animals is ind cated

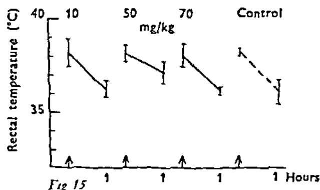


Fig 15

Figs 15, 16 and 17 Rectal temperatures of guinea pigs pretreated with phenobarbital (10, 50 and 70 mg/kg) and of the corresponding control animals in thermal neutrality, and the pentobarbital concentrations in their liver and brain Other data as for Figs 12, 13 and 14

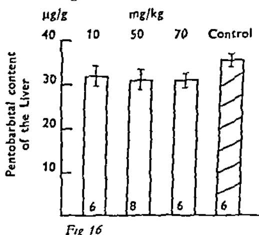


Fig 16

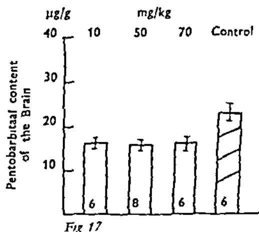


Fig 17

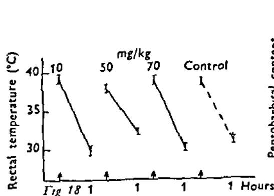


Fig 18

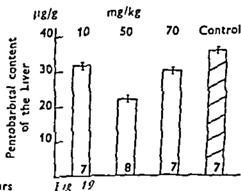


Fig 19

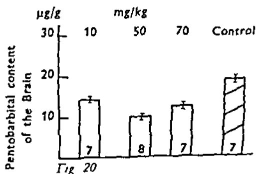


Fig 20

Figs 18 19 and 20 Rectal temperatures of guinea pigs pretreated with phenobarbital (10, 50 and 70 mg kg) and of the corresponding control animals in the cold environment, and the pentobarbital concentrations in their liver and brain Other data as for Figs 12, 13 and 14

than in the group pretreated with 50 mg/kg The pentobarbital concentrations in the brain of the relevant control animals at thermal neutrality and in the cold environment were 23 6±3 4 and 18 3±3 1 μg/g

The pentobarbital concentrations in the liver of the pretreated animals (dose of pretreatment, 50 mg/kg phenobarbital) in the hot environment were identical to those of the corresponding control animals, while at thermal neutrality they were 85 per cent and in the cold environment 62 per cent of the corresponding concentrations of the control animals The pentobarbital concentrations in the brain of these same guinea pigs were, in the hot environment, identical to, but at thermal neutrality 78 per cent and in the cold environment 60 per cent of, the levels of the corresponding control animals In control animals a period of 48 hours in the hot environment slowed down the fall in pentobarbital level by 3 per cent and a similar period at thermal neutrality by 5 per cent, whereas 48 hours in the hot environment accelerated the fall in concentration by 13 per cent, compared with the concentrations of guinea pigs in the acute experiment (Fig 3) The pentobarbital concentrations in the brain of these same control animals were equally high in the hot and cold environments, while in the animals kept at thermal neutrality a 20 per cent rise had taken place in the concentration compared with the levels of the animals in the acute experiment (Fig 5)

Duration of the effect of pretreatment with phenobarbital The accelerating effect of pretreatment with phenobarbital on the biotransformation of pentobarbital emerged clearly in the cold and the thermally neutral environments The pentobarbital concentrations in liver and brain were lower in the pretreated animals, and the change in rectal temperature due to pentobarbital was smaller than in the corresponding control animals In contrast, in the guinea pigs in the hot environment the pretreatment with phenobarbital was not found to have accelerated the biotransformation of pentobarbital For this reason the duration of the inducing effect was only studied at the cold and neutral temperatures

The results are given in Figs 21—26 which indicate the rectal temperatures of pretreated guinea pigs after 48, 72 and 120 hours and of the corresponding control animals, as well as the pentobarbital concentrations in all these animals liver and brain studied from specimens taken one hour after pentobarbital injection

Rectal temperature At thermal neutrality the fall in rectal temperature under pentobarbital action was smallest in the animals pretreated more than 48 hours earlier In the control animals, and in those pretreated more than 48 hours previously, the fall in rectal temperature

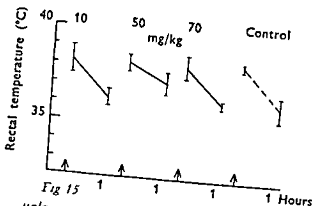


Fig 15

Figs 15, 16 and 17 R
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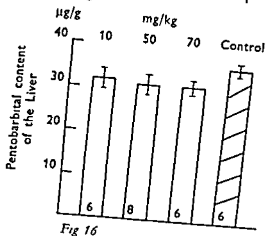


Fig 16

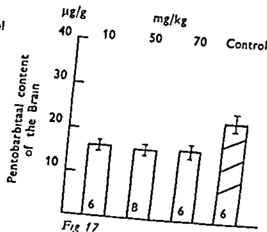


Fig 17

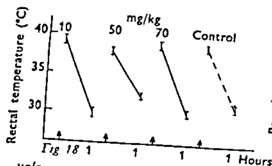


Fig 18

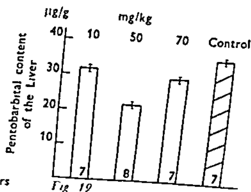


Fig 19

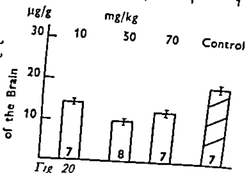


Fig 20

Figs 18 19 and 20 Rectal temperatures of guinea pigs pre treated with phenobarbital (10, 50 and 70 mg kg) and of the corresponding control animals in the cold environment, and the pentobarbital concentrations in their liver and brain Other data as for Figs 12, 13 and 14

was more pronounced In the cold environment the changes in the pretreated animals under pentobarbital action were similar though more pronounced The control animals in the cold environment showed a more marked change in rectal temperature than the pretreated animals

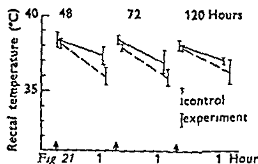
Pentobarbital concentrations in the liver At thermal neutrality, the pentobarbital concentrations in the liver were lowest among the guinea pigs pretreated 48 hours previously, although the concentrations of those pretreated even earlier were on approximately the same level In the corresponding control animals the pentobarbital concentrations of the liver fell within 120 hours In the cold environment, the concentrations of the animals pretreated 48 hours previously were lower than those of the corresponding control animals In the animals pretreated even earlier the concentrations rose to equal those of the control animals

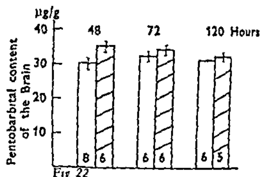
Pentobarbital concentrations in the brain In the neutral temperature conditions the pentobarbital concentrations in the brain of all the pretreated animals were of the same order In the cold environment the lowest concentrations were seen in the animals pretreated 48 hours previously, whereas in those pretreated earlier the concentrations rose to equal the pentobarbital concentrations in the brain of the control animals

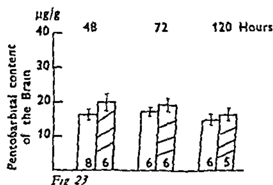
DISCUSSION

In guinea pigs pretreated with phenobarbital the accelerated biotransformation of pentobarbital manifested itself in the steeper fall of the pentobarbital concentrations in liver and brain than in control animals The result was a slighter fall in rectal temperature under pentobarbital action in the pretreated than in the control animals

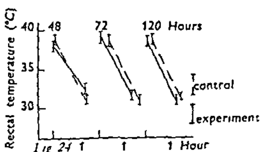
The effect of pretreatment was comparatively most pronounced in the cold environment Yet the accelerated biotransformation of pentobarbital and the reduced fall in rectal temperature due to pentobarbital action were not the result of phenobarbital pretreatment alone The animals not pretreated also showed accelerated fall in cold environment in tissue levels of pentobarbital and retarded fall in rectal temperature compared with the *animals in the* one hour test (Figs 2 3 and 4) A similar observation concerning the accelerating effect of the cold environment on hydroxylation of acetanilide has been reported before (Inscoe & Axelrod 1960) The accelerating effect of a cold environment on drug metabolism can be explained on the basis of increased secretion of adrenal cortex hormones, their

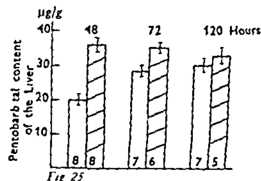


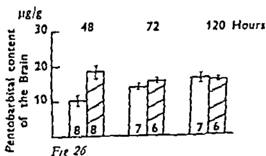




Figs 21, 22 and 23 Rectal temperatures of guinea pigs pretreated with phenobarbital (50 mg/kg) and of the corresponding control animals, in thermal neutrality, and the pentobarbital concentrations in their liver and brain, one hour after the injection of pentobarbital (30 mg/kg) The pentobarbital was injected 48, 72 and 120 hours after pretreatment Mean ± S E M The number of animals in indicated







Figs 24 25 and 26 Rectal temperatures of guinea pigs pretreated with phenobarbital (50 mg/kg) and of the corresponding control animals, in cold environment, and the pentobarbital concentrations in their liver and brain one hour after the injection of pentobarbital Other data as for Figs 21 22 and 23

sponding control animals, the pentobarbital concentrations in the liver of pretreated animals were also clearly lower in thermal neutrality That pretreatment in thermal neutrality was less effective than in the cold environment, appears contradictory Earlier authors have found that the guinea pig feels best under thermally neutral conditions and needs the least amount of energy to maintain its organic functions (HERRINGTON 1940) Hence it would be reasonable to presume that the pretreatment effect would emerge most markedly at the neutral temperature, since accelerated biotransformation of drugs is based on increased synthesis of enzymes in the liver (e g, REMMER 1959 a, CONNEY & BURNS 1959) Thus when the metabolism of the animal is not burdened by efforts to maintain body temperature within the normal range, the induction effect in the pretreated animals could be expected to emerge most clearly An explanation is found in the pentobarbital concentrations in the liver and brain of the control animals living at thermal neutrality these concentrations were higher than in the animals staying an hour at a corresponding temperature (Figs 6 and 7) Not until 120 hours under thermally neutral conditions were the pentobarbital concentrations in the liver and brain of the control animals the same as those of the animals in the 1-hour test The normal environmental temperature in the stables of the guinea pig strain employed for the present studies had been +18-+22°C, and a prolonged stay at thermal neutrality would therefore subject the animals to a slight 'thermal stress" This assumption is supported by the fact that no induction effect emerged in the hot environment and that the pentobarbital concentrations in the liver and brain of both the pretreated and control animals were equally high and, moreover, higher than those of the guinea pigs participating in the 1 hour test (Figs 9 and 10) In the hot environment the guinea pigs' thermal regulation functions poorly (HERRINGTON 1940) and body temperature tends to increase Apparently, with the increase in body temperature, enzyme formation in the guinea pigs was inhibited, and for this reason the biotransformation of pentobarbital was impaired in the animals in the hot environment On the other hand, remaining at a temperature different from the normal environment causes stress in the animals In the hot environment, only the secretion of antidiuretic hormone and aldosterone has been found to have increased (HELLMAN et al 1956, FLETCHER et al 1961) Administration of mineralocorticoids for test purposes has not been found to exert a stimulating effect on the biotransformation of drugs (REMMER 1958 a, b) and therefore neither »warmth» nor »heat» stress stimulates pentobarbital biotransformation as »cold» stress does For this reason pretreatment with phenobarbital

administration to control animal as pretreatment has been found to stimulate enzymatic biotransformation of drugs (REMMER 1957 1958a b) In states of stress — and a period in the cold environment can be considered stress — the duration of the action of hexobarbital pentobarbital and meprobamate in the rat has been found to diminish (RUPE et al 1963) In guinea pigs the secretion of 17 hydroxycorticoids has been found to increase by more than 10 times during neurogenic stress (NIEMELA et al 1960 1962) and under cold temperature conditions to twice the normal level (D ANGELO 1960) Apparently the accelerated pentobarbital biotransformation seen in the control animals of the present study was a result of the inducing effect of adrenal corticosteroids on the hepatic enzymes catalyzing drug metabolism while in the pretreated guinea pigs the »cold stress» and phenobarbital together induced hepatic enzymes Thus pentobarbital biotransformation was accelerated The stimulating effect of phenobarbital on pentobarbital metabolism decreased over 120 hours at the end of which in the pretreated animals the pentobarbital concentrations in the liver and brain and the fall in rectal temperature under pentobarbital action reached the levels of the corresponding control animals

In the cold environment the guinea pigs pretreated with a 50 mg/kg dose of phenobarbital showed the most markedly accelerated liver decomposition of pentobarbital In guinea pigs pretreated with smaller or larger doses of phenobarbital the decomposition of pentobarbital was slower and the fall in rectal temperature due to the pentobarbital more pronounced Apparently a dose of 10 mg phenobarbital per kg bodyweight is too small to induce enzyme formation in the liver of the guinea pig The guinea pigs pretreated with 70 mg/kg had due to drug action a hypothermic period of 5—6 hours which apparently retarded the emergence of the induction effect When a larger pretreatment dose of phenobarbital 100 mg/kg was given all the guinea pigs included in this test died within 4—5 hours with their rectal temperatures falling sharply Furthermore in guinea pigs with a pretreatment dose of 70 mg/kg retention of phenobarbital in the tissues was noted at the end of 48 hours after pentobarbital injection these animals showed a more pronounced fall in rectal temperature as a result of a combined effect of phenobarbital and pentobarbital on the thermal regulation centres This heavier fall in rectal temperature in turn retarded the biotransformation of pentobarbital in the liver tissue of these animals

In the thermally neutral range the accelerating effect of pretreatment with phenobarbital on the biotransformation of pentobarbital emerged less clearly than in the cold environment Compared with the corre

sponding control animals, the pentobarbital concentrations in the liver of pretreated animals were also clearly lower in thermal neutrality That pretreatment in thermal neutrality was less effective than in the cold environment appears contradictory Earlier authors have found that the guinea pig feels best under thermally neutral conditions and needs the least amount of energy to maintain its organic functions (HERRINGTON 1940) Hence it would be reasonable to presume that the pretreatment effect would emerge most markedly at the neutral temperature since accelerated biotransformation of drugs is based on increased synthesis of enzymes in the liver (e g, REMMER 1959 a, CONNEY & BURNS 1959) Thus when the metabolism of the animal is not burdened by efforts to maintain body temperature within the normal range, the induction effect in the pretreated animals could be expected to emerge most clearly An explanation is found in the pentobarbital concentrations in the liver and brain of the control animals living at thermal neutrality these concentrations were higher than in the animals staying an hour at a corresponding temperature (Figs 6 and 7) Not until 120 hours under thermally neutral conditions were the pentobarbital concentrations in the liver and brain of the control animals the same as those of the animals in the 1 hour test The normal environmental temperature in the stables of the guinea pig strain employed for the present studies had been +18 +22°C, and a prolonged stay at thermal neutrality would therefore subject the animals to a slight thermal stress' This assumption is supported by the fact that no induction effect emerged in the hot environment and that the pentobarbital concentrations in the liver and brain of both the pretreated and control animals were equally high and, moreover, higher than those of the guinea pigs participating in the 1 hour test (Figs 9 and 10) In the hot environment the guinea pigs' thermal regulation functions poorly (HERRINGTON 1940) and body temperature tends to increase Apparently, with the increase in body temperature, enzyme formation in the guinea pigs was inhibited, and for this reason the biotransformation of pentobarbital was impaired in the animals in the hot environment On the other hand, remaining at a temperature different from the normal environment causes stress in the animals In the hot environment, only the secretion of antidiuretic hormone and aldosterone has been found to have increased (HELLMAN et al 1956, FLETCHER et al 1961) Administration of mineralocorticoids for test purposes has not been found to exert a stimulating effect on the biotransformation of drugs (REMMER 1958 a, b) and therefore neither »warmth» nor »heat» stress stimulates pentobarbital biotransformation as »cold» stress does For this reason pretreatment with phenobarbital

at thermal neutrality produced only a slight effect on the acceleration of pentobarbital biotransformation, and in the hot environment the decomposition of pentobarbital was not found to accelerate in the pretreated animals In the literature no attention is paid to the part played by environmental temperature in studies of the induction effect

At thermal neutrality, retention of phenobarbital in the tissues had occurred in the course of 48 hours in guinea pigs pretreated with a dose of 70 mg/kg, and for this reason the group, under pentobarbital action, showed a more marked fall in the rectal temperature than that pretreated with a smaller dose of phenobarbital At the hot temperature, too, phenobarbital had remained in the tissues of pretreated animals although small doses were used, and for this reason they showed a more marked increase in rectal temperature under pentobarbital action than the corresponding control animals

The animals' weight fell during the experiments in all thermal conditions, the change in weight of the pretreated animals was in each temperature group the same as that shown by the controls In the hot environment the animals' weight fell by 40—47 g, in thermally neutral conditions by 5—7 g and in the cold environment by 20—30 g During the period of pretreatment the animals had free access to fresh food and water in their cages, consequently the weight loss was not caused by restricted availability of food or drink, both of which have been found to affect the biotransformation of drugs (BORCELLECA & MANTHEI 1957) Apparently life in an environment differing from the thermal conditions in the stables was such a stress on the guinea pigs that their body weights fell while the organism made efforts to adapt itself to the prevailing temperature The weight loss was not correlated to the fall in the tissue levels of pentobarbital at different temperatures

VIII. Effect of enzyme inhibitors at different temperatures

SKF-525 A and JB-516 were chosen as inhibitors of the biotrans formation of pentobarbital in the present studies because they are both wellknown, and most commonly used in comparable experiments The animals remained for one hour in their respective thermal conditions, after which the inhibitor was injected Pentobarbital was injected one hour later The criteria used to indicate the effectiveness of the inhibitory action were change in the rectal temperature due to pentobarbital action in the pretreated guinea pigs, and an analysis of the pentobarbital concentrations in liver and brain specimens taken 2 hours after the injection of pentobarbital

RESULTS

PRETREATMENT WITH SKF 525 A

The pretreatment doses of SKF 525 A at all temperatures were 10, 50 and 100 mg/kg

Results The results are given in Figs 27—35, which specify the rectal temperatures of the pretreated and the relevant control animals subjected to pentobarbital, and the pentobarbital concentrations in liver and brain specimens taken from the animals 2 hours after the injection of pentobarbital

Rectal temperature The pretreatment with SKF 525 A in itself had an inhibiting effect on the thermogenesis of the guinea pig, and the pretreated animals had lower rectal temperatures than the control animals Under pentobarbital action the pretreated animals in all thermal conditions showed a more pronounced change in rectal temperature than the corresponding control animals The rectal temperature of the pretreated animals in the hot environment was 39 1—40 3°C, in thermal neutrality 35 4—37 2°C, and in cold environment 15 0—22 1°C, the figures for the control animals were 40 0—41 0°C, 35 6—37 1°C, and 23 6—31 4°C, respectively In all thermal environments the rectal temperature was lowest in the guinea pigs pretreated with a dose of 100 mg/kg

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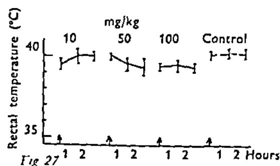


Fig 27

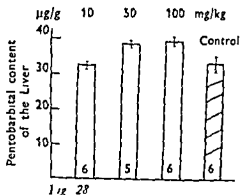


Fig 28

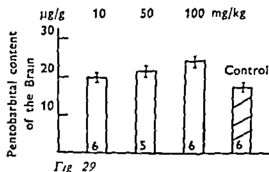


Fig 29

Figs 27, 28 and 29 Rectal temperatures of guinea pigs pretreated with SKF-525 A (10, 50 and 100 mg/kg) and of the corresponding control animals, in the hot environment, and the pentobarbital concentrations in their liver and brain two hours after intraperitoneal injection of pentobarbital The pentobarbital was injected one hour after pretreatment Mean ± SEM The number of animals is indicated

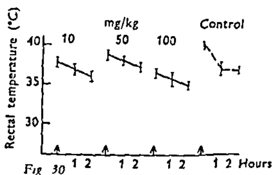


Fig 30

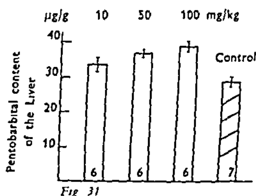


Fig 31

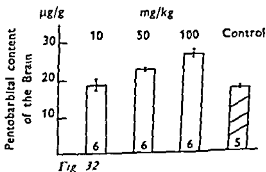


Fig 32

Figs 30 31 and 32 Rectal temperatures of guinea pigs pretreated with SKF 525 A (10, 50 and 100 mg kg) and of the corresponding control animals, in thermal neutrality, and the pentobarbital concentrations in their liver and brain two hours after intraperitoneal injection of pentobarbital Other data as for Figs 27, 28 and 29

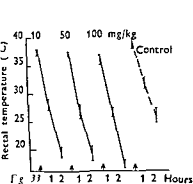


Fig 33

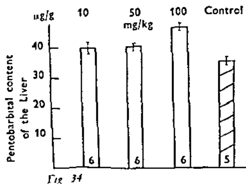


Fig 34

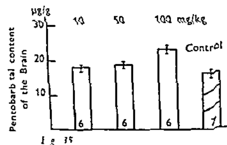


Fig 35

Figs 33 34 and 35 Rectal temperatures of guinea pigs pretreated with SKF 525 A and of the corresponding control animals, in cold environment, and the pentobarbital concentrations in their liver and brain two hours after intraperitoneal injection of pentobarbital Other data as for Figs 27, 28 and 29

Liver and brain concentrations of pentobarbital The pentobarbital concentrations in the liver of the animals pretreated with the highest dose (SKF 525 A 100 mg/kg) were in the hot environment 39 8+2 5 μg/g at thermal neutrality 38 1±4 5 μg/g and in the cold environment 46 3±3 5 μg/g In animals pretreated with a smaller dose the pentobarbital concentrations in the liver were lower The pentobarbital concentrations of the corresponding control animals were 36 1±4 2 μg/g 27 9±5 2 μg/g and 35 8±4 5 μg/g The concentrations in the brain of pretreated animals (SKF 525 A 100 mg/kg) were in the hot environment 24 8±3 6 μg/g at thermal neutrality 22 6±3 9 μg/g and in the cold environment 24 3+2 6 μg/g In animals pretreated with a smaller dose the pentobarbital concentrations in the brain were lower The pentobarbital concentration of the corresponding control animals were 17 2+2 5 μg/g, 16 5±3 1 μg/g and 17 6±3 2 μg/g The relative effectivity of pretreatment with SKF 525 A in inhibiting the fall in the pentobarbital contents of the liver and brain as compared with those of the corresponding control animals is presented in Table 1

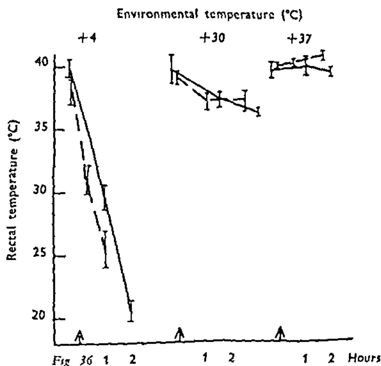
Table 1 Pentobarbital concentrations in the liver and brain of guinea pigs pretreated with SKF 525 A as compared with the corresponding concentrations in control animals (per cent)

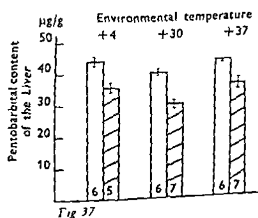
SKF 525 A mg/kg	Environmental temperature +4°C Liver	+4°C Brain	+30°C Liver	+30°C Brain	+37°C Liver	+37°C Brain
Control	100	100	100	100	100	100
10	112	113	128	110	97	115
50	128	130	131	112	108	126
100	134	142	137	137	110	144

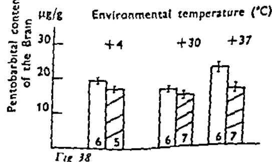
PRETREATMENT WITH JB-516

The pretreatment dose of JB-516 at all temperatures was 15 mg/kg, some animals were pretreated at thermal neutrality with a dose of 3 mg/kg.

Results. The results are given in Figs. 36—38, which specify the rectal temperatures of the pretreated and the corresponding control animals, and the pentobarbital concentrations in liver and brain tissue on the basis of specimens taken 2 hours after the injection of pentobarbital.







Figs 36, 37 and 38 Rectal temperatures of guinea pigs pretreated with JB-516 (15 ... control animals, in different environmental tempera- ... in their liver and brain two hours after ... he pentobarbital was injected one hour after pretrea... of animals is indicated

Rectal temperature The rectal temperature of the pretreated animals in the hot environment was 39 6—40 1°C, at thermal neutrality 35 9—36 9°C and in the cold environment 18 8—22 7°C The rectal temperatures of the corresponding control animals were 40 1—40 9°C, 37 1—37 7°C and 24 0—28 1°C, respectively

The pentobarbital concentrations in the liver of the pretreated animals were in the hot environment 43 4±3 3 μg/g, at thermal neutrality 40 1±3 1 μg/g, and in cold environment 43 5±2 1 μg/g. The corresponding concentrations in the control animals were 36 2±5 7 μg/g, 29 8±4 7 μg/g and 35 1±3 2 μg/g These concentrations in the pretreated animals were in the hot environment 118 per cent, at thermal neutrality 135 per cent and in the cold environment 123 per cent of the corresponding concentrations of the control animals

The pentobarbital concentrations in the brain of the pretreated guinea pigs were in the hot environment 23 3±3 3 μg/g, at thermal neutrality 17 0±2 1 μg/g and in cold environment 19 2±2 3 μg/g The figures for the control animals were 18 7±2 0 μg/g, 16 1±3 1 μg/g, and 18 3±2 5 μg/g The pentobarbital concentrations in the brain of the pretreated animals were in the hot environment 125 per cent, at thermal neutrality 105 per cent and in cold environment 105 per cent of the corresponding concentrations in control animals

At thermal neutrality the pentobarbital concentration of the liver of guinea pigs pretreated with the small dose was 35 5±1 5 μg/g

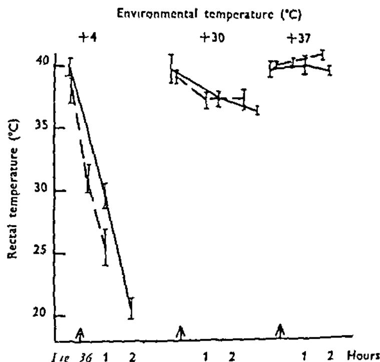
DISCUSSION

Both SKF 525 A and JB 516 are effective and widely used agents to inhibit biotransformation of drugs in the liver (e g, NETTER 1962) It has been shown that SKF 535 A is relatively more capable than JB-516 of inhibiting biotransformation (SERRONE & FUJIMOTO 1962) The difference between these drugs has not been only quantitative JB 516 is merely an inhibiting agent whereas with SKF-525 A, after the inhibitory effect has disappeared, a slight acceleration of biotransformation may be noted (KATO et al 1964) In addition, in rats pretreated with JB-516 the duration of the action of thiopenthal is extended (SERRONE & FUJIMOTO 1962), whereas with SKF 525 A it is not altered (COOK et al 1954 a)

From the present results, environmental temperature may be said to affect the guinea pigs pretreated with SKF 525 A more markedly than those pretreated with JB 516 Both these inhibitors prevented the fall in the pentobarbital concentration of the liver JB 516, however, was relatively more effective in inhibiting

The pretreatment dose of JB-516 at all temperatures was 15 mg/kg, some animals were pretreated at thermal neutrality with a dose of 3 mg/kg.

Results. The results are given in Figs. 36—38, which specify the rectal temperatures of the pretreated and the corresponding control animals, and the pentobarbital concentrations in liver and brain tissue on the basis of specimens taken 2 hours after the injection of pentobarbital.



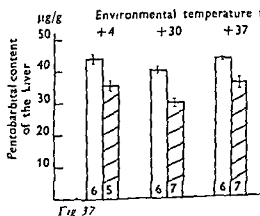


Fig 37

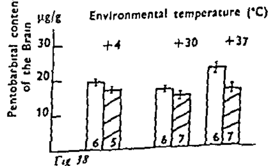


Fig 38

Figs 36, 37 and 38 Rectal temperatures of guinea pigs pretreated with JB 516 (15 mg/kg) and of the corresponding control ... temperatures, and the pentobarbital concentration ... ours after intraperitoneal injection of pentobarbital ... our after pretreatment Mean ± S E M The numb

was lower than that of the control animals At thermal neutrality the rectal temperature of the pretreated declined during the second hour, unlike the controls In the cold environment both in the pretreated and control animals the fall in rectal temperature was linear and heavy

When the liver pentobarbital concentrations of the pretreated and control animals are compared, the effect of any change in body temperature on the biotransformation of pentobarbital should be taken into account Pentobarbital induced a heavier temperature fall in the pretreated than the control animals, with resulting inhibition of the biotransformation of pentobarbital

In hot environment the rectal temperature of the pretreated guinea pigs increased less than that of control animals, in the latter, therefore, the retarding effect of the hot environment on the biotransformation of pentobarbital appeared more marked

In many studies the durations of sleeping times have been recorded to provide a measure of the biotransformation of barbiturates Pentobarbital enters brain tissue better in hot than cold environments When the decomposition of the drug in the liver is inhibited by enzyme inhibitors relatively more of the active pentobarbital remains in the brain tissue in the hot than in the cold environment Consequently, extension of sleeping time in hot environment does not only imply retarded metabolism, but the effect of temperature on the distribution between tissues must also be taken into account

the effect of environmental temperature on the fall of the pentobarbital concentration in the liver In the pretreated animals, the pentobarbital concentrations in the liver were at the same level in all temperatures In the animals pretreated with SKF-525 A, on the other hand, the inhibition of the biotransformation of pentobarbital depended on environmental temperature In earlier studies, as small a dose of SKF-525A as 1 mg/kg has been found to extend the sleeping time significantly after an intraperitoneal injection of hexobarbital (COOK et al 1954 a) According to the present results, not even a relatively large dose, 100 mg/kg, of the inhibitor could inhibit the influence of the environmental temperature on the biotransformation of pentobarbital In the animals pretreated with SKF-525 A the fall in the pentobarbital concentration of the liver was relatively most effectively inhibited at thermal neutrality, where the fall in the concentration in control animals was heaviest In the cold environment the pentobarbital concentrations in the liver of control animals were high probably due to a slow biotransformation For this reason the inhibitory effect of SKF 525 A was slighter at the cold than at the neutral temperature In the hot environment pretreatment with SKF-525 A was relatively least effective in inhibiting the fall in the pentobarbital concentration of the liver A contributory factor may be the fact that the rectal temperature of animals pretreated with SKF-525 A was lower than that of the corresponding control animals Consequently, the retarding effect of heat on the biotransformation of pentobarbital was not as intense as in the control animals

Pentobarbital enters the brain tissue of animals better in a hot than in a cold environment (SETNIKAR & TEMELCOU 1962) The concentration in the brain remained higher at the hot than at the other temperatures When a large dose of SKF 525 A was used the pentobarbital concentration was higher in the brain than in the liver In other words, pretreatment with enzyme inhibitor affected the distribution of pentobarbital between brain and liver tissues in the hot environment in favour of the former This may be partly responsible for the fact that in guinea pigs pretreated with SKF 525 A the inhibition of the fall in the pentobarbital concentration in the liver appeared to be relatively smaller in the hot than in the other environments

Both SKF-525 A and JB-516 inhibit the thermogenesis of the guinea pigs, the rectal temperature of pretreated animals was lower than that of the control animals After the injection of pentobarbital the change in the rectal temperature of the guinea pigs pretreated with inhibitor was more pronounced than that in the corresponding control animals In the hot environment the rectal temperature of the pretreated

was lower than that of the control animals At thermal neutrality the rectal temperature of the pretreated declined during the second hour, unlike the controls In the cold environment both in the pretreated and control animals the fall in rectal temperature was linear and heavy.

When the liver pentobarbital concentrations of the pretreated and control animals are compared, the effect of any change in body temperature on the biotransformation of pentobarbital should be taken into account Pentobarbital induced a heavier temperature fall in the pretreated than the control animals, with resulting inhibition of the biotransformation of pentobarbital

In hot environment the rectal temperature of the pretreated guinea pigs increased less than that of control animals, in the latter, therefore, the retarding effect of the hot environment on the biotransformation of pentobarbital appeared more marked

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Both SKF 525 A and JB 516 inhibit the thermogenesis of the guinea pigs the rectal temperature of pretreated animals was lower than that of the control animals After the injection of pentobarbital the change in the rectal temperature of the guinea pigs pretreated with inhibitor was more pronounced than that in the corresponding control animals In the hot environment the rectal temperature of the pretreated

Table 2 Effect of environmental temperature on the rectal temperatures and the barbital concentrations in the liver and brain, of guinea pigs pretreated with phenobarbital (50 mg/kg) and of the corresponding control animals.

Environmental temperature (°C)	Pretreatment	No of animals	Rectal temperature after 3 hrs (°C±S.E.M.)	Barbital concentrations (µg/g±S.E.M.) Liver	Brain
+4	Control	7	23.6±2.0	120.1±3.1	110.0±4.1
	Phenobarbital	7	22.4±1.0	119.0±2.2	109.0±6.0
+30	Control	7	36.1±0.5	118.6±2.3	113.0±5.2
	Phenobarbital	7	36.7±0.6	113.2±4.1	105.6±3.6
+37	Control	6	38.9±1.0	117.8±2.0	111.4±4.2
	Phenobarbital	6	38.6±0.8	117.1±3.1	111.3±4.8

Table 3 Effect of environmental temperature on the rectal temperatures, and the barbital concentrations in the liver and brain of guinea pigs pretreated with SKF 525 A (50 mg/kg) and JB 516 (15 mg kg) and of the corresponding control animals

Environmental temperature (°C)	Pretreatment	No of animals	Rectal temperature after 3 hrs (°C±S.E.M.)	Barbital concentrations (µg/g±S.E.M.) Liver	Brain
+4	Control	7	19.9±1.2	118.8±3.2	108.1±1.8
	SKF 525 A	7	22.3±3.0	116.6±2.3	104.4±4.0
	JB 516	7	18.7±1.2	118.2±3.3	112.0±3.5
+30	Control	7	36.5±1.6	116.3±1.0	108.3±3.0
	SKF 525 A	7	37.0±0.6	109.1±2.1	105.4±6.0
	JB 516	7	36.3±0.4	116.1±2.1	110.0±6.5
+37	Control	7	40.6±0.3	112.1±1.2	106.0±1.7
	SKF 525 A	7	40.3±0.4	109.7±1.7	110.0±4.0
	JB 516	7	40.4±0.3	112.3±2.6	109.1±3.4

temperature of the animals treated with barbital was in hot, neutral and cold environments similar to that in guinea pigs subjected to the influence of pentobarbital after pretreatment with enzyme inhibitors. Since practically all barbital is excreted unchanged into the urine, individual differences in the activity of the liver enzymes cannot affect the barbital content of the tissues. The result was a uniform rectal temperature change in the animals treated with barbital due to the action of the barbital. In the guinea pigs pretreated with drugs inhibiting or inducing the microsomal enzymes of the liver, the fall in the rectal temperature was similar to that in the animals treated only with barbital.

Barbital concentration in the liver and brain. In earlier studies the drugs which induce (Remmer 1959 a) or inhibit (Cook et al 1954 a,

IX. Effect of environmental temperature on the fall of barbital concentrations in liver and brain

In the preceding chapter environmental temperature was found to affect the biotransformation of pentobarbital, metabolized in the liver Barbital on the other hand is a drug almost all of which is excreted unchanged into the urine The effect of barbital on the guinea pig's rectal temperature, as well as its concentration in the liver and brain tissues were studied Also the effect of pretreatment with phenobarbital, SKF-525 A and JB-516 was studied The treatment of the animals and the management of the experiments were described in the preceding chapter The doses used for pretreatment were phenobarbital 50 mg/kg, SKF-525 A 50 mg/kg and JB-516 15 mg/kg

RESULTS

The results are given in Tables 2 and 3, which list the guinea pigs' rectal temperatures, and the barbital concentrations in the liver and brain specimens taken 3 hours after the injection of barbital

Rectal temperature The change in the rectal temperature of the pretreated guinea pigs was similar to that of the animals treated only with barbital

Barbital concentration in the liver and brain Both in animals given the inducing and the inhibiting pretreatment the barbital concentrations in the liver and brain were identical to those of the animals treated only with barbital

DISCUSSION

Rectal temperature Barbital, 250 mg per kg of body weight, put the guinea pigs into a deep sleep, making their body temperature dependent on the environmental temperature The change in rectal

Table 2 Effect of environmental temperature on the rectal temperatures and the bar bital concentrations in the liver and brain of guinea pigs pretreated with phenobarbital (50 mg/kg) and of the corresponding control animals

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				Liver	Brain
+4	Control	7	23 6±2 0	120 1±3 1	110 0±4 1
	Phenobarbital	7	22 4±1 0	119 0±2 2	109 0±6 0
+30	Control	7	36 1±0 5	118 6±2.3	113 0±5 2
	Phenobarb tal	7	36 7±0 6	113 2±4 1	105 6±3 6
+37	Control	6	38 9±1 0	117 8±2 0	111 4±4.2
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				Liver	Brain
+4	Control	7	19 9±1.2	118 8±3 2	108 1±1 8
	SKF 525 A	7	22.3±3 0	116 6±2 3	104 4±4 0
	JB 516	7	18 7±1.2	118.2±3.3	112 0±3.5
+30	Control	7	36 5±1 6	116.3±1 0	108 3±3 0
	SKF 525 A	7	37 0±0 6	109 1±2 1	105 4±6 0
	JB 516	7	36 3±0 4	116 1±2 1	110.0±6 5
+37	Control	7	40 6±0 3	112 1±1 2	106 0±1 7
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	JB 516	7	40 4±0 3	112.3±2 6	109 1±3 4

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Barbital concentration in the liver and brain Both in animals given the inducing and the inhibiting pretreatment the barbital concentrations in the liver and brain were identical to those of the animals treated only with barbital

DISCUSSION

Rectal temperature Barbital, 250 mg per kg of body weight put the guinea pigs into a deep sleep, making their body temperature dependent on the environmental temperature The change in rectal

X. General discussion

The role of temperature in studies of the basic properties of drugs has not been sufficiently emphasized The majority of drugs are metabolized enzymatically in the organism If body temperature fluctuates beyond the normal range enzyme activity is affected *In vitro* studies have shown the importance of temperature for the biotransformation of drugs (RINK et al 1956, LEADBEATER & DAVIES 1964), whereas in *in vivo* studies, which still form the basis of experimental pharmacological research the effect of temperature on the biotransformation of drugs has been almost completely ignored For example, information on the chronic toxicity of new drugs is first obtained from test animal studies If adequate attention is not given to checks of environmental temperature, the results obtained may be erroneous

In the present study, the test animal was the guinea pig since earlier studies (GOESSLIN 1949) have shown the guinea pig to be more sensitive to the influence of environmental temperature than e g the rat which has been used most often in previous experimental studies The initial intention of this study was to analyse the barbiturate concentrations in the liver, brain and plasma However, technical problems arose owing to the difficulty of obtaining sufficient blood samples from guinea pigs with body temperatures below 30 0°C A similar finding has been recorded from examination of larger mammals, too, for example, it is difficult to obtain blood specimens from a hypothermic human being (HARKINS & HARMON 1934) This is due to the fact that fluid penetrates the blood vessels into the interstitial tissue and the circulating blood volume is reduced In the rat, the haematocrit value in hypothermia increases from 42 up to 65—70 per cent (POPOVIC & KENT 1965)

From the present experiments, environmental temperature can be said to affect the biotransformation *in vivo* of pentobarbital which is metabolized in the liver, but not the fall during short term tests in the tissue concentrations of barbital which is excreted unchanged The

NETTER 1962) the enzymes of the liver were found to affect the sleeping time of the animals after barbital injection According to the present results, inhibiting or inducing pretreatment did not affect the fall in the barbital concentration of the liver and brain in different thermal environments during a short term experiment Irrespective of environmental temperature the liver and brain concentrations in the pretreated and control animals were similar Nor was the change in the guinea pigs' body temperature, under the action of barbital found to correlate, during a 3 hour test, to the fall in the barbital concentration in the tissues, whereas a correlation between body temperature and tissue concentrations was found under the action of pentobarbital metabolized in the liver The results concur with those reported earlier in the literature the duration of sleeping time in experimental animals treated with a barbital injection is independent of body temperature (FUHRMAN 1947), and the fall in the barbital level of blood in a short term test is independent of body temperature (SETNIKAR & TEMELCOU 1962)

X. General discussion

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rate of fall in the pentobarbital concentrations in the liver and brain is better correlated to body temperature than environmental temperature This is because the change in guinea pigs' body temperature due to the action of pentobarbital is not uniform in all individuals, there is even considerable individual fluctuation Both rise and fall in body temperature have a reatarding influence on biotransformation of pentobarbital Environmental temperature also plays a part in the onset of the enzyme induction produced by phenobarbital In the guinea pigs of the present series the inducing effect, measured by the fall in pentobarbital concentrations of the liver and brain and in animals under the influence of pentobarbital, by the intensity of the change in rectal temperature, was strongest in the cold environment At thermal neutrality the effect of pretreatment with phenobarbital emerged less clearly, and at the hot temperature no effect was produced The inhibiting effect of SKF 525 A on the biotransformation of pentobarbital depended on the temperature, whereas that of JB 516 emerged with the same intensity in all thermal environments

Earlier studies have shown that sleep caused by short-acting, but not that caused by long acting, barbiturates was prolonged in a cold environment In addition, agents lowering the body temperature have been found to extend the duration of sleep produced by short acting barbiturates (LESSIN & PARKES 1957) The extended duration of the effect has been considered a sign of retarded biotransformation of barbiturates in the organism (FUHRMAN 1947) In the present study, pentobarbital but not barbital was found to enter the brain tissue more easily in the hot than the cold environment, an observation made earlier (e g, SETNIKAR & TEMELCOU 1962) On the other hand, in the hot environment animals have been found to wake up with higher barbiturate concentrations than in the cold environment (WINNIE 1964), and the duration of sleep has not always been found to correlate to the barbiturate content of the brain (SMITH 1961) For this reason, only measuring the duration of sleep caused by barbiturates cannot be considered a reliable method for the study of the biotransformation of barbiturates at different temperatures

Environmental temperatures vary greatly in the different seasons of the year, especially in the cold and temperate climatic zones Laboratory temperatures in the different parts of the world are probably relatively accurately regulated within the range 18—23°C But in animal stables, without adequate temperature regulation, temperatures may fall in the cold and rise in the hot seasons The temperature of the animals growth environment may affect study results Furthermore, one stable may have a low temperature and another a higher temperature, and

although the animals are transferred to the same laboratory temperature for the duration of the experiments, the response of their body temperature during the tests will be different (SCHWABE et al 1938) This may affect the results of the test On the basis of the above, it may be called into question whether studies of drug metabolism carried out with test animals in different parts of the world are mutually comparable The part played by environmental temperature is emphasized in studies of drugs which affect the regulation of the animal's body temperature and make *the small animal almost poikilothermic* To ensure that temperatures do not affect the results obtained with test animals, the tests should be carried out separately in each climatic zone, or detailed instructions be issued to make the living conditions of test animals identical In the present study of course the temperatures in the test rooms differed so heavily that such differences could hardly occur without special arrangements in ordinary laboratory work Hence it is important to check body temperatures during experiments, even in ordinary laboratory conditions

The study arouses an interesting question of whether environmental temperature also affects human drug metabolism Man is homothermic, but in different thermal zones he has to adapt himself to different conditions In addition, environmental temperatures fluctuate inside any one climatic zone from season to season, and with present fast traffic connections trips are frequently made from one climatic zone to the other In diseases in which body temperature rises, e g infections, human drug metabolism may undergo changes, which could play a part should the drug employed produce an »undesired effect» The posology of drugs for man in different climatic zones has been insufficiently studied, as pointed out by WEIHE (1965)

XI. Summary

The purpose of the present work was to study the effect of environmental temperature (a) on the rectal temperature of the guinea pig, (b) on the fall in the liver and brain concentrations of pentobarbital metabolized in the organism and of barbital excreted unchanged, (c) on the induction of the microsomal enzymes by pretreatment with phenobarbital in the light of the fall in the tissue concentrations of pentobarbital and barbital, (d) on the inhibition of microsomal enzymes by SKF-525 A and JB-516 in the light of the fall in the tissue concentrations of pentobarbital and barbital

(1) A change in environmental temperature was found to affect the rectal temperature of the guinea pigs In a cold environment (+4°C) the rectal temperatures remained within the normal range throughout the test In thermally neutral environment (+30°C) the rectal temperature rose at first, but the guinea pig adapted itself in 3—4 hours, and the rectal temperature fell into the normal range In the hot environment (+37°C) the rectal temperature rose fast to 40—41°C and, within 6 hours, the guinea pig was unable to normalize its temperature

(2) Under the action of pentobarbital the guinea pig's rectal temperature was still more susceptible to environmental temperature In the cold environment the rectal temperature fell markedly in all animals, the change was correlated to the dose of drug given At the same time, marked individual dispersal occurred in the fall of rectal temperature In neutral thermal environment it fell in 2 hours by 2—3°C, but in the third hour it moved towards the initial level In hot environment the rectal temperature remained in the range of 40—42°C

(3) The disappearance of pentobarbital in 1, 2 and 3 hours from the liver tissue was correlated to the change in the guinea pig's body temperature The disappearance was quickest in the animals whose rectal temperature was closest to the normal range, whereas the fall of rectal temperature to 26° uniformly retarded the disappearance of

pentobarbital from liver tissue Once the temperature fell below 26°C no biotransformation of pentobarbital in liver tissue took place The rise of rectal temperature in hot environment also slowed down the disappearance of pentobarbital from liver tissue

(4) The pentobarbital content of the brain in cold and neutral thermal environment was correlated to the corresponding fall in the liver content In the hot environment the brain concentrations were relatively higher than the corresponding liver concentration would have suggested, when the mean pentobarbital contents of the brain were compared with the values for animals kept at a cold or a neutral temperature

(5) The induction effect produced by pretreatment with phenobarbital was manifested in accelerated fall in the liver and brain concentrations of pentobarbital and in retarded fall of rectal temperature The effect was most pronounced in the cold environment At the neutral temperature the induction effect was weaker, and in hot environment no such effect emerged at all

(6) Keeping the guinea pigs for 48—120 hours in the cold environment accelerated the fall in the tissue concentrations of pentobarbital, whereas the neutral and hot environments slowed down this fall

(7) The effect of SKF 525 A inhibiting microsomal enzymes was manifested in retarded disappearance of pentobarbital, and in a marked fall in rectal temperature during pentobarbital anaesthesia The pretreatment had the most pronounced inhibiting effect on the biotransformation of pentobarbital at the neutral temperature, where the animals not pretreated exhibited the fastest disappearance of pentobarbital from the tissue While the decomposition of pentobarbital in liver tissue was inhibited, rectal temperature fell evenly in the neutral and cold environments In the cold, in particular, individual dispersal in the fall of rectal temperature was not noted In the hot environment pretreatment with SKF 525 A was relatively least effective When the decomposition of pentobarbital in the liver tissue was inhibited the brain level of pentobarbital rose relatively most in the hot environment

(8) The effect of JB 516, which also inhibits microsomal enzymes, manifested itself in a rise in the tissue concentrations of pentobarbital and in a marked fall of rectal temperature under anaesthesia JB-516 was a relatively more effective inhibitor for the guinea pig than SKF-525 A at different environmental temperatures The liver concentrations of pentobarbital in animals pretreated with JB-516 were equally high in the hot and the cold environments, and at the neutral temperature the level did not significantly differ from the

other two While the decomposition of pentobarbital in the liver was inhibited, the brain concentration increased relatively heavily in the hot environment

(9) Under the action of barbital the rectal temperature of the guinea pig fell in the cold environment within 3 hours to below 20°C Individual dispersal was slight In the neutral environment the rectal temperature fell in 3 hours uniformly by 2—3°C In the hot environment the rectal temperature rose, exceeding 40°C In guinea pigs pretreated with enzyme inhibitors, JB 516 or SKF 525 A, the changes in rectal temperature in different environmental temperatures were similar to those in the control animals In those pretreated with enzyme inducing phenobarbital, in 48 hours the fall in rectal temperature under barbital anaesthesia was similar to that in the corresponding control animals

(10) In 3 hours environmental temperatures did not affect the fall of liver and brain concentrations of barbital, which is excreted unchanged In the cold, neutral and hot environments the barbital concentrations in the tissues were the same Neither inhibiting nor inducing pretreatment caused any fall in the barbital content of the liver and brain different from that in the animals treated with barbital

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